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INDIAN AGRICULTURAL  
RESEARCH INSTITUTE, NEW DELHI.







# JOURNAL

OF THE

## ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

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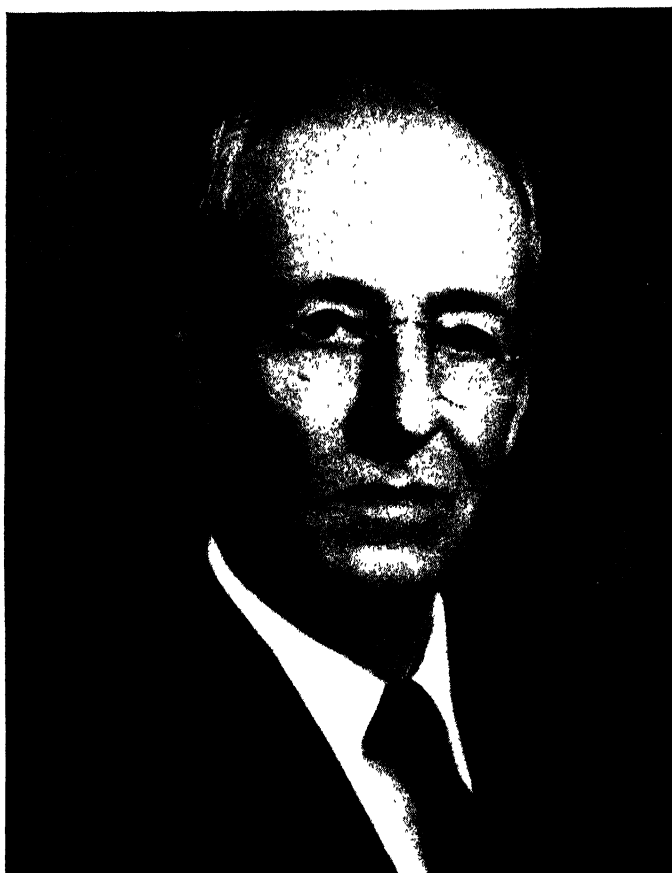
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ELNATHAN KEMPER NELSON, 1870 1940

## ELNATHAN KEMPER NELSON

The death of E. K. Nelson, Senior Chemist of the Agricultural Chemical Research Division of the Bureau of Agricultural Chemistry and Engineering, at his home in Silver Spring, Md., on November 9, after a brief illness, came as a profound shock to many of his friends and associates. He would have reached the age of 70 on November 25, and his passing thus coincided almost with the date of his retirement from official work.

Nelson was born at Cincinnati on November 25, 1870; he obtained his B.S. degree in chemistry at the University of Illinois in 1894. For the next 15 years he broadened his experience by working as chemist in various Chicago packing-house industries and as a private consultant. In 1909 he joined the staff of the Bureau of Chemistry and was Chief of its Essential Oils Laboratory until 1927, when he became Senior Chemist of the Food Research Division of the newly reorganized Bureau of Chemistry and Soils.

Nelson's first attendance at a meeting of the Association of Official Agricultural Chemists was at the twenty-seventh convention in 1910, when he presented a paper on the "Quantitative Determination of Ketones in Essential Oils."<sup>1</sup> From this time until the fifty-fifth convention in 1939 he never missed a meeting of the Association. At the 1912 convention Nelson read a "Report on the Determination of Camphor by the Hydroxylamin Method,"<sup>2</sup> and at the 1914 meeting a paper on the "Determination of Santonin in Levant Wormwood."<sup>3</sup>

At the 1923 meeting of the Association Nelson was appointed Associate Referee on Fruit Acids, on which subject he presented reports at the 40th,<sup>4</sup> 41st,<sup>5</sup> 42nd,<sup>6</sup> and 43rd<sup>7</sup> meetings. At the 41st meeting he presented also a paper<sup>8</sup> on the "Detection and Determination of Lactic Acid in the Presence of Other Organic Acids." At the 44th convention in 1929 Nelson presented a "Report on Chenopodium Oil,"<sup>9</sup> of which product he did much to elucidate the composition and on which he published numerous papers for over a period of 18 years. His work on essential oils gave him marked prominence in this field of investigation. At the 44th meeting Nelson was appointed General Referee on Canned Foods, but other engagements prevented him from performing the duties of this office.

At the 1935 meeting of the Association Nelson presented a "Report on Oil of Peppermint."<sup>10</sup> His last contribution to the work of the Association was a joint contribution by A. L. Curl and himself, read at the 1938 meeting, on "Observations on the Colorimetric Method for Vanillin."<sup>11</sup>

The papers presented before this Association represent only a few of Nelson's numerous contributions on the chemistry of the rarer constituents of foods and condiments. These articles were published in circulars and bulletins of the Bureau of Chemistry, in the *Year Books* of the Department of Agriculture, in the various journals of the American Chemical Society, in the *Journal of the American Pharmaceutical Association*, in *Science*, in the *American Journal of Physiology*, in the *Fruit Products Journal*, in the *American Perfumer and Essential Oil Review*, in *Food Industries*, and in other miscellaneous publications. It is regrettable that Nelson could not have lived to write up in permanent monograph form the results of many of his scattered phytochemical researches on capsacin, ascaridol, pectin,

<sup>1</sup> Bur. Chem. Bull. 137, pp. 186-187.

<sup>2</sup> *Ibid.*, 162, pp. 208-209.

<sup>3</sup> *This Journal*, 2, 79-82 (1916).

<sup>4</sup> *Ibid.*, 8, 637-640 (1925).

<sup>5</sup> *Ibid.*, 9, 375-382 (1926).

<sup>6</sup> *Ibid.*, 10, 432-433 (1927).

<sup>7</sup> *Ibid.*, 11, 444-445 (1928).

<sup>8</sup> *Ibid.*, 9, 331-333 (1926).

<sup>9</sup> *Ibid.*, 12, 303-304 (1929).

<sup>10</sup> *Ibid.*, 19, 529-532 (1936).

<sup>11</sup> *Ibid.*, 22, 684-688 (1939).

and flavoring substances and on the organic acids of fruits and vegetables and of various manufactured food products. He won world-wide recognition for his work on the isolation and identification of the rarer acids and other constituents of plant materials. His investigation of glucic acid, a puzzle to chemists for over a century, was especially noteworthy, as he was the first to isolate it in a pure form. He called attention to the remarkable reducing properties of this substance, which was afterwards renamed "reducton" by Euler.

Nelson's connection of nearly 32 years with the food research work of the Department of Agriculture was marked by the publication of over 70 papers, of which it is impossible to give a detailed account in the present sketch. Among his last important assignments was an investigation on the chemistry of vanilla beans, which he conducted in Puerto Rico in 1938, at the request of the Agricultural Experiment Station of that island.

Mr. Nelson is survived by his widow, Mrs. Blanche Parker Nelson; two sons, Elnathan Kemper Nelson, Jr., of Wheeling, West Virginia, and Capt. Berkley E. Nelson, of Kelly Field; and a brother, Major Frank L. Nelson, U. S. A., retired, of Fort Benning, Georgia. "E.K.," as he was affectionately called by the inner circle of his friends, will long be remembered by the members of our Association, not only for his fundamental contributions to the science of plant chemistry but for his constant adherence to the highest ethical standards of his profession. In all his relations he exemplified perfectly the old Horatian precept—"*Integer vitae scelerisque purus*"—

CHARLES A. BROWNE.

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**PROCEEDINGS OF THE FIFTY-SIXTH ANNUAL  
CONVENTION OF THE ASSOCIATION OF  
OFFICIAL AGRICULTURAL  
CHEMISTS, 1940**

The fifty-sixth annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., October 28, 29, and 30, 1940.

The meeting was called to order by the president, W. W. Skinner, Bureau of Agricultural Chemistry and Engineering, Washington, D. C., on the morning of October 28, at 10:30 o'clock.

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**OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE  
REFEREES OF THE ASSOCIATION OF OFFICIAL  
AGRICULTURAL CHEMISTS FOR THE YEAR  
ENDING NOVEMBER, 1941**

*President*

L. B. BROUGHTON, University of Maryland, College Park, Md.

*Vice-President*

J. W. SALE, U. S. Food and Drug Administration,  
Washington, D. C.

*Secretary-Treasurer*

W. W. SKINNER, Bureau of Agricultural Chemistry and  
Engineering, Washington, D. C.

*Additional Members of the Executive Committee*

G. G. FRARY, Vermillion, S. D.

J. O. CLARKE, Chicago, Ill.

G. H. MARSH, Montgomery, Ala.

**PERMANENT COMMITTEES**

*Recommendations of Referees*

(Figures in parentheses refer to year in which appointment expires.)

HENRY A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.),  
*Chairman*

SUBCOMMITTEE A: H. A. Halvorson (1942) (Department of Agriculture, Dairy and Food, St. Paul, Minn.), *Chairman*; E. L. GRIFFIN (1944), and G. E. GRATTAN (1946).

Enzymes (papain)

Feeding stuffs

Sampling

Ash

Mineral mixed feeds (calcium and  
iodine)

Lactose in mixed feeds

Manganese

Carotene and cryptoxanthin in yellow  
corn

Crude and pure carotene

Fat in fish meal

Adulterants of condensed milk products and cod-liver oil	Plants
Starch	Sampling
Fat in cooked animal feeds containing cereals	Iodine and boron
Crude fat or ether extract	Carbohydrates
Filtration aids in crude fiber determination	Zinc and iron
Salt	Copper and cobalt
Ammoniacal urea and nitrogen salts	Chlorophyl and carotene
Fertilizers	Hydrocyanic acid
Phosphoric acid (moisture)	Soils and liming materials
Nitrogen	H-ion concentration of soils of arid and semi-arid regions
Magnesium and manganese	Fluorine
Acid and base-forming quality	Boron in soils
Potash	Zinc in soils
Calcium and sulfur	Exchangeable hydrogen in soils
Copper and zinc	Liming materials
Platinum recovery methods	Standard solutions
Insecticides and fungicides	Constant boiling hydrochloric acid
Total fluorine	Sodium thiosulfate solutions
Silicofluoride in admixture with sodium fluoride	Thiocyanate solutions
Napthalene in poultry lice products	Sulfuric acid
Leathers and tanning materials	Vitamins
Paints, paint materials, and varnishes	Vitamin A
Accelerating testing of paints	Vitamin B <sub>1</sub>
Varnishes	Vitamin D—milk
	Vitamin D for poultry
	Vitamin K
	Riboflavin
SUBCOMMITTEE B: A. E. PAUL (1942) (U. S. Food and Drug Administration, Chicago, Ill.), <i>Chairman</i> ; W. F. REINDOLLAR (1944), and H. J. FISHER (1946).	
Naval stores	Sulfapyridine
Radioactivity	Metrazol
Vegetable drugs and their derivatives	Sulfathiazol
Chemical methods for ergot alkaloids	Applicability of the present method for barbital and phenobarbital to other barbiturates
Theophyllin sodium salicylate	Aminopyrine and ephedrine
Physostigmine in ointments	Miscellaneous drugs
Arecoline hydrobromide	Microchemical tests for alkaloids and synthetics
Quinine ethyl carbonate	Magnesium trisilicate
Theobromine and phenobarbital	Determination of mercury compounds by the ethanolamine method
Plasmochine	Separation of bromides, chlorides, and iodides
Cinnamyl ephedrine	Thyroid
Ascorbic acid	Ethyl and isopropyl alcohols and acetone
Prostigmine	Emulsions
Polarograph methods for drugs	Compound ointment of benzoic acid
Synthetic drugs	Bromide preparations
Benzedrine	
Hydroxyquinoline sulfate	
Methylene blue	
Aminopyrine, acetophenetidin, caffeine, and phenobarbital	
Ethyl aminobenzoate	

**Drug bioassays****Cosmetics and coal-tar colors**

Moisture in cosmetics  
 Common ash constituents  
 Alkalies in cuticle removers  
 Arsenic in hair lotions  
 Lead in cosmetics  
 Mercury salts in cosmetics  
 Peroxides in cosmetics  
 $\beta$ -naphthol in hair lotions  
 Pyrogallol in hair dyes  
 Resorcinol in hair lotions  
 Salicylic acid in hair lotions  
 Cosmetic creams  
 Cosmetic powders  
 Dentifrices and mouth washes  
 Deodorants and anti-perspirants  
 Depilatories  
 Hair dyes and rinses  
 Hair straighteners

**Lip make-up and rouges**

Lotions for eye and skin  
 Mascara, eyebrow pencils, and eye shadow  
 Nail cosmetics  
 Ether extract in coal-tar colors  
 Pure dye, impurities, and substrata in pigments  
 Buffers and solvents in titanium trichloride titration  
 Halogens in halogenated fluoresceins  
 Intermediates in certified coal-tar colors  
 Spectrophotometric testing of coal-tar colors  
 Subsidiary dyes in D & C colors  
 Identification of certified coal-tar colors  
 Alizarin and madder lake

**SUBCOMMITTEE C:** W. B. WHITE (1942) (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*; J. O. CLARKE (1944), and C. S. LADD (1946).

**Canned foods**

Tomato products  
 Quality factors and fill of container

**Coffee and tea**

Chlorogenic acid in coffee

**Coloring matters in foods****Dairy products**

Neutralizers  
 Decomposition  
 Fat in butter  
 Mold mycelia in butter  
 Lactose in milk  
 Pasteurization of milk and cream  
 Total solids and ash in milk and evaporated milk  
 Fat in malted milk  
 Casein in malted milk  
 Lactic acid in dried and skim milk  
 Sugar in sweetened condensed milk  
 Cheese (isolation and identification of fat)  
 Sampling cheese  
 Frozen desserts

**Eggs and egg products**

Unsaponifiable matter and cholesterol  
 Fat and acidity of ether extract  
 Decomposition  
 Added glycerol

**Fish and other marine products****Total solids and ether extract****Ash, salt, and total nitrogen****Ammonia****Volatile acids****Formic acid in canned salmon and tuna fish****Gums in foods**

Soft curd cheese  
 Mayonnaise and French dressing  
 Frozen desserts  
 Starchy foods

**Meat and meat products****Dried skim milk and soy bean flour****Metals in foods**

Arsenic and antimony  
 Copper  
 Zinc  
 Fluorine  
 Lead  
 Mercury  
 Selenium  
 Hydrocyanic acid

**Microbiological methods****Canned fishery products****Canned meats****Canned vegetables****Canned tomatoes and other acid vegetables and fruit products****Sugar**

Frozen egg products  
 Frozen fruits and vegetables  
 Nuts and nut products  
 Microchemical methods  
 Oils, fats, and waxes  
   Refractometric determination of oil  
   in seeds  
   Polenske method  
   Olive oil in admixture with other oils  
   Titer test  
   Unaponifiable matter  
 Preservatives and artificial sweeteners  
   Saccharin in non-alcoholic beverages,

semi-solid preparations, and baked  
 goods  
 Benzoate of soda  
 Esters of benzoic acid  
 Spices and condiments  
   Vinegar  
   Salad dressings  
   Volatile oil in spices  
   Moisture and ash in spices  
   Ash, salt, and starch in prepared mus-  
   tard; starch in mustard flour; and  
   volatile oil in mustard seed

SUBCOMMITTEE D: J. W. SALE (1942) (U. S. Food and Drug Administration, Wash-  
 ington, D. C.), *Chairman*; J. A. LeCLERC (1944), and W. C. JONES (1946).

Alcoholic beverages  
   Diastatic activity of malt  
   Proteolytic activity of malt  
   Malt extract in malt  
   Malt adjuncts  
   Hops  
   Beer  
   Iron in beer  
   Tin and copper in beer  
   Carbon dioxide in beer  
   Sulfur dioxide in beer and wine  
   Volatile acidity of wines  
   Methanol in distilled spirits  
   Denaturants in distilled spirits  
   Whiskey and rum  
   Cordials and liqueurs  
 Cacao products  
   Lecithin in cacao products  
   Pectin acid in cacao products  
   Milk protein in milk chocolate  
   Chocolate constituents  
 Cereal foods  
   Hydrogen-ion concentration  
   Starch in raw and cooked cereals  
   Sugar in bread and other cereal prod-  
   ucts  
   Milk solids in bread  
   Butterfat in bread  
   Rye flour in rye bread and in flour  
   mixtures  
   Moisture, ash, protein, fat, and crude  
   fiber in baked products  
   Fat acidity in grain, flour, corn meal,  
   and whole wheat flour  
   Baking test for soft wheat flour  
   Chlorine in fat of flour

Benzoyl peroxide in flour  
 Carotenoid pigments in flour  
 Carbon dioxide in self-rising flour  
 Proteolytic activity of flour  
 Cellulose in whole wheat flour prod-  
 ucts  
 Moisture in self-rising flour and in  
 pancake, waffle, and doughnut  
 flours  
 Phosphated flour  
 Soya flour in foods  
 Oats  
 Corn  
 Rye and buckwheat  
 Rice and barley  
 Macaroni products  
 Noodles  
 Flavors and non-alcoholic beverages  
    $\beta$ -ionone  
   Lemon oils and extracts  
   Organic solvents in flavors  
   Glycerol, vanillin, and coumarin in  
   vanilla and imitation vanillas  
   Emulsion flavors  
   Maple flavor concentrates and imita-  
   tions  
 Fruits and fruit products  
   Sodium and chlorides  
   Polariscopic methods  
   Electrometric titration of acids  
   Fruit acids  
   Phosphoric acid ( $P_2O_5$ )  
   Potassium  
     (a) Cobaltinitrite procedure  
     (b) Rapid control method  
   Cold pack fruits

Sugars and sugar products	Honey and honey dew honey
Unfermented reducing substances in molasses	Sucrose and ash in molasses
Diacetyl	Refractive indices of sugar solutions
Drying, densimetric, and refractometric methods	Waters, brine, and salt
	Fluorine and boron in water

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SAMPLING:

L. M. Jeffers, Dept. of Agriculture, Sacramento, Calif.

ASH:

J. L. St. John, Agricultural Experiment Station, Pullman, Wash.

MINERAL MIXED FEEDS (CALCIUM AND IODINE):

A. T. Perkins, Kansas State College, Manhattan, Kan.

LACTOSE IN MIXED FEEDS:

D. A. Magraw, American Dry Milk Inst., Chicago, Ill.

MANGANESE:

J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

CAROTENE AND CRYPTOXANTHIN IN YELLOW CORN:

A. R. Kemmerer, Agricultural Experiment Station, College Station, Tex.

CRUDE AND PURE CAROTENE:

A. R. Kemmerer

FAT IN FISH MEAL:

R. W. Harrison, Bureau of Fisheries, Seattle, Wash.

ADULTERATION OF CONDENSED MILK PRODUCTS AND COD-LIVER OIL:

P. B. Curtis, Agricultural Experiment Station, Lafayette, Ind.

STARCH:

P. B. Curtis.

FAT IN COOKED ANIMAL FEEDS CONTAINING CEREALS:

J. D. Turner, Agricultural Experiment Station, Lexington, Ky.

**CRUDE FAT OR ETHER EXTRACT:**

J. J. Taylor, Dept. of Agriculture, Tallahassee, Fla.

**FILTRATION AIDS IN CRUDE FIBER DETERMINATION:**  

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**SALT:**

J. W. Kuzmeski, Agricultural Experiment Station, Amherst, Mass.

**AMMONIACAL UREA AND NITROGEN SALTS:**

W. B. Griem, Dept. of Agriculture, Madison, Wis.

**FERTILIZERS:**

*Referee:* G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

**PHOSPHORIC ACID (MOISTURE):**

W. H. Ross, Bureau of Plant Industry, Washington, D. C.

**NITROGEN:**

A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

**MAGNESIUM AND MANGANESE:**

J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

**ACID AND BASE-FORMING QUALITY:**

H. R. Allen, Agricultural Experiment Station, Lexington, Ky.

**POTASH:**

O. W. Ford, Agricultural Experiment Station, Lafayette, Ind.

**CALCIUM AND SULFUR:**

Gordon Hart, Dept. of Agriculture, Tallahassee, Fla.

**COPPER AND ZINC:**

W. Y. Gary, Dept. of Agriculture, Tallahassee, Fla.

**PLATINUM RECOVERY METHODS:**

O. W. Ford

**INSECTICIDES AND FUNGICIDES:**

*Referee:* J. J. T. Graham, Agricultural Marketing Service, Washington, D. C.

**TOTAL FLUORINE:**

C. G. Donovan, Agricultural Marketing Service, Washington, D. C.

**SILICOFLUORIDE IN ADMIXTURE WITH SODIUM FLUORIDE:**

C. G. Donovan

**NAPHTHALENE IN POULTRY LICE PRODUCTS:**

Rosewell Jinkins, Food and Drug Administration, Chicago, Ill.

**LEATHERS AND TANNING MATERIALS:**

*Referee:* I. D. Clarke, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

**PAINTS, PAINT MATERIALS, AND VARNISHES:**

*Referee:* C. S. Ladd, State Regulatory Laboratory, Bismarck, N. D.

**ACCELERATING TESTING OF PAINTS:**

L. L. Carrick, Agricultural Experiment Station, Fargo, N. D.

**VARNISHES:**

F. Roberts, Paint and Varnish Laboratory, Bismarck, N. D.

**PLANTS:**

*Referee:* E. J. Miller, Agricultural Experiment Station, East Lansing, Mich.

**SAMPLING:**

E. J. Miller

**IODINE AND BORON:**

J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

**CARBOHYDRATES:**

J. T. Sullivan, U. S. Regional Pasture Research Lab., State College, Pa.

**ZINC AND IRON:**

Hale Cowling, Agricultural Experiment Station, East Lansing, Mich.

**COPPER AND COBALT:**

Lillian I. Butler, Agricultural Experiment Station, East Lansing, Mich.

**CHLOROPHYL AND CAROTENE:**

E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.

**HYDROCYANIC ACID:**

R. A. Greene, University of Arizona, Tucson, Ariz.

**SOILS AND LIMING MATERIALS:**

*Referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

**HYDROGEN-ION CONCENTRATION OF SOILS OF ARID AND SEMI-ARID REGIONS:**

W. T. McGeorge, Agricultural Experiment Station, Tucson, Ariz.

**BORON AND FLUORINE IN SOILS:**

J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

**ZINC IN SOILS:**

Lewis H. Rogers, 222 University Ave., Ithaca, N. Y.

**LIMING MATERIALS:**

W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

**EXCHANGEABLE HYDROGEN IN SOILS:**

W. M. Shaw

**STANDARD SOLUTIONS:**

*Referee:* R. L. Vandaveer, Food and Drug Administration, New Orleans, La.

**CONSTANT BOILING HYDROCHLORIC ACID:**

W. H. King, Food and Drug Administration, New Orleans, La.

**SODIUM THIOSULFATE SOLUTIONS:**

G. M. Johnson, Food and Drug Administration, St. Louis, Mo.

**THIOCYANATE SOLUTIONS:**

E. C. Deal, Food and Drug Administration, New Orleans, La.

**SULFURIC ACID:**

Harry Conroy, Food and Drug Administration, Kansas City, Mo.

**VITAMINS:**

*Referee:* E. M. Nelson, Food and Drug Administration, Washington, D. C.

**VITAMIN A:**

J. B. Wilkie, Food and Drug Administration, Washington, D. C.

**VITAMIN B<sub>1</sub>:**

O. L. Kline, Food and Drug Administration, Washington, D. C.

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W. C. Russell, Agricultural Experiment Station, New Brunswick, N. J.

**VITAMIN D—POULTRY:**

C. D. Tolle, Food and Drug Administration, Washington, D. C.

**VITAMIN K:**

H. J. Almquist, University of California, Berkeley, Calif.

**RIBOFLAVIN:**

A. R. Kemmerer, Agricultural Experiment Station, College Station, Tex.

*Subcommittee B***NAVAL STORES:**

*Referee:* V. E. Grotlisch, Agricultural Marketing Service, Washington, D. C.

**RADIOACTIVITY:**

*Referee:* A. Wolf, Food and Drug Administration, Washington, D. C.

**VEGETABLE DRUGS AND THEIR DERIVATIVES:**

*Referee:* F. H. Wiley, Food and Drug Administration, Washington, D. C.

**CHEMICAL METHODS FOR ERGOT ALKALOIDS:**

D. C. Grove, Food and Drug Administration, Washington, D. C.

**THEOPHYLLIN SODIUM SALICYLATE:**

M. Harris, Food and Drug Administration, Houston, Tex.

**PHYSOSTIGMINE IN OINTMENTS:**

G. M. Johnson, Food and Drug Administration, St. Louis, Mo.

**ARECOLINE HYDROBROMIDE:**

H. R. Bond, Food and Drug Administration, Kansas City, Mo.

**QUININE ETHYL CARBONATE:**

H. G. Underwood, Food and Drug Administration, Chicago, Ill.

**THEOBROMINE AND PHENOBARBITAL:**

E. C. Deal, Food and Drug Administration, New Orleans, La.

**PLASMOCHINE:**

F. C. Sinton, Food and Drug Administration, New York City

**CINNAMYL EPHEDRINE:**

J. Claggett Jones, 1123 State Office Bldg., Richmond, Va.

**ASCORBIC ACID:**

J. J. Taylor, Department of Agriculture, Tallahassee, Fla.

**PROSTIGMINE:**

F. J. McNall, Food and Drug Administration, Cincinnati, Ohio.

**POLAROGRAPH METHODS:**

S. Resnek, Food and Drug Administration, New York City.

**SYNTHETIC DRUGS:**

*Referee:* L. E. Warren, Food and Drug Administration, Washington, D. C.

**BENZEDRINE:**

J. H. Cannon, Food and Drug Administration, St. Louis, Mo.

**HYDROXYQUINOLINE SULFATE:**

A. W. Allison, Food and Drug Administration, Buffalo, N. Y.

**METHYLENE BLUE:**

H. O. Moraw, Food and Drug Administration, Chicago, Ill.

**AMINOPYRINE, ACETOPHENETIDIN, CAFFEINE, AND PHENOBARBITAL:**

Jonas Carol, Food and Drug Administration, Chicago, Ill.

**ETHYL AMINO BENZOATE:**

J. R. Matchett, Bureau of Internal Revenue, Washington, D. C.

**SULFAPYRIDINE:**

I. Schurman, Food and Drug Administration, Cincinnati, Ohio.

**METRAZOL:**

S. M. Berman, Food and Drug Administration, Buffalo, N. Y.

**SULFATHIAZOL:**

Herzl Cohen, Food and Drug Administration, Chicago, Ill.

**APPLICABILITY OF PRESENT METHOD FOR BARBITAL AND PHENOBARBITAL TO  
OTHER BARBITURATES:**

L. E. Warren

**AMINOPYRINE AND EPHEDRINE:**

Harry Rogavitz, Food and Drug Administration, New York City.

**MISCELLANEOUS DRUGS:**

*Referee:* C. K. Glycart, Food and Drug Administration, Chicago, Ill.

**MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS:**

G. L. Keenan, Food and Drug Administration, Washington, D. C.

**MAGNESIUM TRISILICATE:**

E. K. Tucker, Dept. of Agriculture and Industry, Montgomery, Ala.

**MERCURY COMPOUNDS (ethanolamine method):**

P. S. Jorgensen, Food and Drug Administration, San Francisco, Calif.

**SEPARATION OF BROMIDES, CHLORIDES, AND IODIDES:**

N. E. Freeman, Food and Drug Administration, Atlanta, Ga.

**THYROID:**

M. L. Yakowitz, Food and Drug Administration, San Francisco, Calif.

**ETHYL AND ISOPROPYL ALCOHOLS AND ACETONE:**

M. Orchiss, Food and Drug Administration, Cincinnati, Ohio.

**EMULSIONS:**

Harold F. O'Keefe, Food and Drug Administration, Chicago, Ill.

**COMPOUND OINTMENT OF BENZOIC ACID:**

W. F. Kunke, Food and Drug Administration, Chicago, Ill.

**BROMIDE PREPARATIONS:**

R. Hyatt, Food and Drug Administration, Cincinnati, Ohio.

**DRUG BIOASSAYS:**

*Referee:* L. C. Miller, Food and Drug Administration, Washington, D. C.

**COSMETICS AND COAL-TAR COLORS:**

*Referee:* Dan Dahle, Food and Drug Administration, Washington, D. C.

**MOISTURE IN COSMETICS:**

Nathan Foote, Food and Drug Administration, Philadelphia, Pa.

**COMMON ASH CONSTITUENTS:**

J. A. Batscha, Food and Drug Administration, New York City

**ALKALIES IN CUTICLE REMOVERS:**

R. E. Duggan, Food and Drug Administration, New Orleans, La.

**ARSENIC IN HAIR LOTIONS:**

H. L. Burrill, Dept. of Health and Welfare, Augusta, Me.

**LEAD IN COSMETICS:**

W. C. Woodfin, Food and Drug Administration, Atlanta, Ga.

**MERCURY SALTS IN COSMETICS:**

S. H. Perlmutter, Food and Drug Administration, Minneapolis, Minn.

**PEROXIDES IN COSMETICS:**

K. F. McClure, Food and Drug Administration, Baltimore, Md.

 **$\beta$ -NAPHTHOL IN HAIR LOTIONS:**

L. O. Weiss, Food and Drug Administration, Los Angeles, Calif.

**PYROGALLOL IN HAIR DYES:**

C. R. Joiner, Food and Drug Administration, New Orleans, La.

**RESORCINOL IN HAIR LOTIONS:**

Frederick M. Garfield, Food and Drug Administration, St. Louis, Mo.

**SALICYLIC ACID IN HAIR LOTIONS:**

H. R. Bond, Food and Drug Administration, Kansas City, Mo.

**COSMETIC CREAMS:**

C. F. Bruening, Food and Drug Administration, Baltimore, Md.

**COSMETIC POWDERS:**

George McClellan, Food and Drug Administration, Washington, D. C.

**DENTIFRICES AND MOUTH WASHES:**

E. H. Grant, Food and Drug Administration, Boston, Mass.

**DEODORANTS AND ANTI-PERSPIRANTS:**

O. C. Maercklein, State Laboratories Dept., Bismarck, N. D.

**DEPILATORIES:**

F. J. McNall, Food and Drug Administration, Cincinnati, Ohio.

**HAIR DYES AND RINSES:**

I. S. Shupe, Food and Drug Administration, Washington, D. C.

**HAIR STRAIGHTENERS:**

J. F. Armstrong, Food and Drug Administration, Los Angeles, Calif.

**LIP MAKE-UP AND ROUGES:**

E. M. Hoshall, Food and Drug Administration, Washington, D. C.

**LOTIONS FOR EYE AND SKIN:**

Norman M. Foster, Food and Drug Administration, Philadelphia, Pa.

**MASCARA, EYEBROW PENCILS, AND EYE SHADOW:**

James W. Fuller, Dept. of Health and Welfare, Augusta, Me.

**NAIL COSMETICS:**

W. H. Naylor, Food and Drug Administration, Seattle, Wash.

**ETHER EXTRACT IN COAL-TAR COLORS:**

S. S. Forrest, Food and Drug Administration, Washington, D. C.

**PURE DYE, IMPURITIES, AND SUBSTRATA IN PIGMENTS:**

G. R. Clark, Food and Drug Administration, Washington, D. C.

**BUFFERS AND SOLVENTS IN TITANIUM TRICHLORIDE TITRATION:**

O. L. Evenson, Food and Drug Administration, Washington, D. C.

**HALOGENS IN HALOGENATED FLUORESCINES:**

J. H. Jones, Food and Drug Administration, Washington, D. C.

**INTERMEDIATES IN CERTIFIED COAL-TAR COLORS:**

S. H. Newburger, Food and Drug Administration, Washington, D. C.

**SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS:**

R. W. Stewart, Food and Drug Administration, Washington, D. C.

**SUBSIDIARY DYES IN D & C COLORS:**

L. Koch, 537 Columbia St., Brooklyn, N. Y.

**IDENTIFICATION OF CERTIFIED COAL-TAR COLORS:**

K. A. Freeman, Food and Drug Administration, Washington, D. C.

**ALIZARIN AND MADDER LAKE:**

W. C. Bainbridge, 537 Columbia St., Brooklyn, N. Y.

*Subcommittee C***CANNED FOODS:**

*Referee:* V. B. Bonney, Food and Drug Administration, Washington, D. C.

**TOMATO PRODUCTS:**

L. M. Beacham, Jr., Food and Drug Administration, Washington, D. C.

**QUALITY FACTORS AND FILL OF CONTAINER:**

S. C. Oglesby, Food and Drug Administration, Washington, D. C.

**COFFEE AND TEA (CHLOROGENIC ACID IN COFFEE):**

*Referee:* H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

**COLORING MATTERS IN FOODS:**

*Referee:* C. F. Jablonski, Food and Drug Administration, New York City.

**DAIRY PRODUCTS:**

*Referee:* G. G. Frary, State Chemical Laboratory, Vermillion, S. D.

**NEUTRALIZERS:**

F. Hillig, Food and Drug Administration, Washington, D. C.

**DECOMPOSITION:**

C. S. Myers, Food and Drug Administration, Washington, D. C.

**FAT IN BUTTER:**

J. A. Mathews, Food and Drug Administration, Washington, D. C.

**MOLD MYCELIA IN BUTTER:**

J. D. Wildman, Food and Drug Administration, Washington, D. C.

**LACTOSE IN MILK:**

E. R. Garrison, University of Missouri, Columbia, Mo.

**PASTEURIZATION OF MILK AND CREAM:**

F. W. Gilcreas, Department of Health, Albany, N. Y.

**TOTAL SOLIDS AND ASH IN MILK AND EVAPORATED MILK:**

G. G. Frary

**MALTED MILK (FAT):**

E. W. Coulter, Food and Drug Administration, Chicago, Ill.



**MALTED MILK (CASEIN):**

I. Schurman, Food and Drug Administration, Chicago, Ill.

**DRIED AND SKIM MILK (LACTIC ACID):**

F. Hillig

**SUGAR IN SWEETENED CONDENSED MILK:**

R. F. Jackson, National Bureau of Standards, Washington, D. C.

**CHEESE (ISOLATION AND IDENTIFICATION OF FAT):**

I. D. Garard, N. J. College for Women, New Brunswick, N. J.

**SAMPLING CHEESE:**

J. B. Snider, Food and Drug Administration, Minneapolis, Minn.

**FROZEN DESSERTS:**

F. Leslie Hart, Food and Drug Administration, Los Angeles, Calif.

**EGGS AND EGG PRODUCTS:**

*Referee:* E. O. Haenni, Food and Drug Administration, Washington, D. C.

**UNSAAPONIFIABLE MATTER AND CHOLESTEROL:**

E. O. Haenni

**FAT AND ACIDITY OF ETHER EXTRACT:**

L. C. Mitchell, Food and Drug Administration, St. Louis, Mo.

**DECOMPOSITION:**

L. C. Mitchell

**ADDED GLYCEROL:**

L. C. Mitchell

**FISH AND OTHER MARINE PRODUCTS:**

*Referee:* H. D. Grigsby, Food and Drug Administration, Philadelphia, Pa.

**TOTAL SOLIDS AND ETHER EXTRACT:**

Manuel Tubis, Food and Drug Administration, Philadelphia, Pa.

**ASH, SALT, AND TOTAL NITROGEN**

H. D. Grigsby

**AMMONIA:**

H. D. Grigsby

**VOLATILE ACIDS:**

F. Hillig, Food and Drug Administration, Washington, D. C.

**FORMIC ACID IN CANNED SALMON AND TUNA FISH:**

F. Hillig

**GUMS IN FOODS:**

*Referee:* F. Leslie Hart, Food and Drug Administration, Los Angeles, Calif.

**SOFT CURD CHEESE:**

M. J. Gnagy, Food and Drug Administration, Los Angeles, Calif.

**MAYONNAISE AND FRENCH DRESSING:**

L. T. Ryan, State Regulatory Laboratory, Bismarck, N. D.

**FROZEN DESSERTS:**

F. Leslie Hart

**STARCHY FOODS:**

Sutton Redfern, Fleischmann Laboratories, New York City.

**MEATS AND MEAT PRODUCTS:**

*Referee:* R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

**DRIED SKIM MILK AND SOY BEAN FLOUR:**

R. H. Kerr

**METALS IN FOODS:**

*Referee:* H. J. Wichmann, Food and Drug Administration, Washington, D. C.

**SELENIUM:**

A. K. Klein, Food and Drug Administration, Washington, D. C.

**ARSENIC AND ANTIMONY:**

A. K. Klein

**COPPER:**

C. A. Greenleaf, National Cannery Association, Washington, D. C.

**ZINC:**

W. S. Ritchie, Agricultural Experiment Station, Amherst, Mass.

**FLUORINE:**

P. A. Clifford, Food and Drug Administration, Washington, D. C.

**LEAD:**

P. A. Clifford

**MERCURY:**

E. P. Lang, Food and Drug Administration, Washington, D. C.

**HYDROCYANIC ACID:**

E. P. Lang

**MICROBIOLOGICAL METHODS:**

*Referee:* A. C. Hunter, Food and Drug Administration, Washington, D. C.

**CANNED FISHERY PRODUCTS:**

O. W. Lang, Hooper Foundation Medical Research, University of California, San Francisco, Calif.

**CANNED MEATS:**

M. L. Laing, Armour and Company, Chicago, Ill.

**CANNED VEGETABLES:**

E. J. Cameron, National Cannery Association, Washington, D. C.

**CANNED TOMATOES AND OTHER ACID VEGETABLES AND FRUIT PRODUCTS:**

B. A. Linden, Food and Drug Administration, Washington, D. C.

**SUGAR:**

E. J. Cameron

**FROZEN EGG PRODUCTS:**

M. T. Bartram, Food and Drug Administration, Washington, D. C.

**FROZEN FRUITS AND VEGETABLES:**

H. E. Goresline, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

**NUTS AND NUT PRODUCTS:**

M. Ostrolenk, Food and Drug Administration, Washington, D. C.

**MICROCHEMICAL METHODS:**

*Referee:* E. P. Clarke, Bureau of Entomology and Plant Quarantine, Washington, D. C.

**OILS, FATS, AND WAXES:**

*Referee:* J. Fitelson, Food and Drug Administration, New York City.

**REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS:**

Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

**POLENSKE METHODS:**

J. A. Mathews, Food and Drug Administration, Washington, D. C.

**OLIVE OIL IN ADMIXTURE WITH OTHER OILS:**

J. Fitelson

**TITER TEST:**

J. Fitelson

**UNSAAPONIFIABLE MATTER:**

Gardner Kirsten, Food and Drug Administration, New York City.

**PRESERVATIVES AND ARTIFICIAL SWEETENERS:**

*Referee:* W. F. Reindollar, Bureau of Chemistry, Department of Health, Baltimore, Md.

**SACCHARIN IN NON-ALCOHOLIC BEVERAGES, SEMI-SOLID PREPARATIONS, AND BAKED GOODS:**

W. F. Reindollar

**ESTERS OF BENZOIC ACID:**

B. G. Hartmann, Food and Drug Administration, Washington, D. C.

**BENZOATE OF SODA:**

A. E. Mix, Food and Drug Administration, Washington, D. C.

**SPICES AND CONDIMENTS:**

*Referee:* S. Alfend, Food and Drug Administration, St. Louis, Mo.

**VINEGAR:**

A. M. Henry, Food and Drug Administration, Atlanta, Ga.

**SALAD DRESSING:**

L. T. Ryan, State Regulatory Laboratory, Bismarck, N. D.

**VOLATILE OIL IN SPICES:**

J. F. Clevenger, Food and Drug Administration, New York City.

**MOISTURE AND ASH IN SPICES:**

S. Alfend

**ASH, SALT, AND STARCH IN PREPARED MUSTARD; STARCH IN MUSTARD FLOUR;  
AND VOLATILE OIL IN MUSTARD SEED:**

J. Thomas Field, Food and Drug Administration, St. Louis, Mo.

*Subcommittee D***ALCOHOLIC BEVERAGES:**

*Referee:* J. W. Sale, Food and Drug Administration, Washington, D. C.

**DIASTATIC ACTIVITY OF MALT:**

Christian Rask, Albert Schwill Co., Chicago, Ill.

**PROTEOLYTIC ACTIVITY OF MALT:**

Stephen Laufer, Schwarz Laboratories, Inc., New York City.

**MALT EXTRACT IN MALT:**

Stephen Jozsa, Fleischmann Malting Co., New York City.

**MALT ADJUNCTS:**

Kurt Becker, Siebel Institute, Chicago, Ill.

**HOPS:**

Frank Rabak, Bureau of Plant Industry, Washington, D. C.

**BEER:**

H. W. Rohde, Schlitz Brewing Co., Milwaukee, Wis.

**IRON IN BEER:**

L. E. Clifcorn, Continental Can Co., Chicago, Ill.

**TIN AND COPPER IN BEER:**

L. E. Clifcorn

**CARBON DIOXIDE IN BEER:**

P. P. Gray, Wallerstein Laboratories, New York City

**SULFUR DIOXIDE IN BEER AND WINE:**

L. V. Taylor, American Can Company, Maywood, Ill.

**VOLATILE ACIDITY OF WINES:**

M. A. Joslyn, Agricultural Experiment Station, Berkeley, Calif.

**METHANOL IN DISTILLED SPIRITS:**

G. F. Beyer, Bureau of Internal Revenue, Washington, D. C.

**DENATURANTS IN DISTILLED SPIRITS:**

G. F. Beyer

**WHISKEY AND RUM:**

Peter Valaer, Bureau of Internal Revenue, Washington, D. C.

**CORDIALS AND LIQUEURS:**

J. B. Wilson, Food and Drug Administration, Washington, D. C.

**CACAO PRODUCTS:**

*Referee:* W. O. Winkler, Food and Drug Administration, Washington, D. C.

**LECITHIN IN CACAO PRODUCTS:**

J. H. Bornmann, Food and Drug Administration, Chicago, Ill.

**PECTIC ACID IN CACAO PRODUCTS:**

W. O. Winkler

**MILK PROTEIN IN MILK CHOCOLATE:**

M. L. Offutt, Food and Drug Administration, New York City.

**CHOCOLATE CONSTITUENTS:**

W. O. Winkler

**CEREAL FOODS:**

*Referee:* V. E. Munsey, Food and Drug Administration, Washington, D. C.

**H-ION CONCENTRATION:**

George Garnatz, Kroger Food Foundation, Cincinnati, Ohio.

**STARCH IN RAW AND COOKED CEREALS:**

M. P. Etheredge, Agricultural Experiment Station, State College, Miss.

**SUGAR IN BREAD AND OTHER CEREAL FOODS:**

R. M. Sanstedt, Agricultural Experiment Station, Lincoln, Neb.

**MILK SOLIDS IN BREAD:**

V. E. Munsey

**BUTTERFAT IN BREAD:**

V. E. Munsey

**RYE FLOUR IN RYE BREAD AND IN FLOUR MIXTURES:**

C. G. Harrel Pillsbury Flour Mills Co., Minneapolis, Minn.

**BAKED PRODUCTS (MOISTURE, ASH, PROTEIN, FAT, AND CRUDE FIBER):**

Stephen Voris, Loose-Wiles Biscuit Co., Long Island City, N. Y.

**FAT ACIDITY IN GRAIN, FLOUR, CORN MEAL, AND WHOLE WHEAT FLOUR:**

Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

**BAKING TEST FOR SOFT WHEAT FLOUR:**

Robert S. Bailey, National Milling Co., Toledo, Ohio.

**CHLORINE IN FAT OF FLOUR:**

Dorothy B. Scott, Food and Drug Administration, New York City.

**BENZOYL PEROXIDE IN FLOUR:**

Dorothy B. Scott

**CAROTENOID PIGMENTS IN FLOUR:**

H. K. Parker, Novadel-Agene Corp., Newark, N. J.

**CARBON DIOXIDE IN SELF-RISING FLOUR:**

R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill.

**PROTEOLYTIC ACTIVITY OF FLOUR:**

Quick Landis, Fleischmann Laboratories, New York City.

**CELLULOSE IN WHOLE WHEAT FLOUR PRODUCTS:**

C. S. Ladd, State Regulatory Laboratory, Bismarck, N. D.

**MOISTURE IN SELF-RISING FLOUR AND IN PANCAKE, WAFFLE, AND DOUGHNUT FLOURS:**

L. H. Bailey, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

**PHOSPHATED FLOUR:**

J. R. Davies, General Foods Corp., Chicago, Ill.

**SOYA FLOUR IN FOODS:**

J. W. Hayward, Archer-Daniels-Midland Co., Milwaukee, Wis.

**OATS:**

H. P. Howells, Quaker Oats Co., Cedar Rapids, Iowa.

**CORN:**

L. R. Brown, A. E. Staley Mfg. Co., Decatur, Ill.

**RYE AND BUCKWHEAT:**

C. G. Harrel

**RICE AND BARLEY:**

Allen D. Dickson, Bureau of Plant Industry, Madison, Wis.

**MACARONI PRODUCTS:**

R. H. Harris, Agricultural Experiment Station, Fargo, N. D.

**NOODLES:**

E. O. Haenni, Food and Drug Administration, Washington, D. C.

**FLAVORS AND NON-ALCOHOLIC BEVERAGES:**

*Referee:* J. B. Wilson, Food and Drug Administration, Washington, D. C.

 **$\beta$ -IONONE:**

J. B. Wilson

**LEMON OILS AND EXTRACTS:**

J. B. Wilson

**ORGANIC SOLVENTS IN FLAVORS:**

R. D. Stanley, Food and Drug Administration, Chicago, Ill.

**GLYCEROL, VANILLIN, AND COUMARIN IN VANILLA AND IMITATION VANILLAS:**

Llewelyn Jones, Food and Drug Administration, Kansas City, Mo.

**EMULSION FLAVORS:**

J. B. Wilson

**MAPLE FLAVOR CONCENTRATES AND IMITATIONS:**

J. L. Perlman, Department of Agriculture, Albany, N. Y.

**FRUITS AND FRUIT PRODUCTS:**

*Referee:* R. A. Osborn, Food and Drug Administration, Washington, D. C.

**SODIUM AND CHLORIDES:**

R. S. Pruitt, Food and Drug Administration, Cincinnati, Ohio.

**POLARISCOPIC METHODS:**

L. H. McRoberts, Food and Drug Administration, San Francisco, Calif.

**ELECTROMETRIC TITRATION OF ACIDS:**

H. M. Bollinger, Food and Drug Administration, San Francisco, Calif.

**FRUIT ACIDS:**

B. G. Hartmann, Food and Drug Administration, Washington, D. C.

**PHOSPHORIC ACID ( $P_2O_5$ ):**

H. Shuman, Food and Drug Administration, Philadelphia, Pa.

**POTASSIUM:**

(a) Cobaltinitrite Procedure: C. A. Wood, Food and Drug Administration, New York.

(b) Rapid Control Method: H. W. Gerrits, Food and Drug Administration, San Francisco, Calif.

**COLD PACK FRUIT:**

Robert Roe, Food and Drug Administration, Seattle, Wash.

**SUGAR AND SUGAR PRODUCTS:**

*Referee:* R. F. Jackson, National Bureau of Standards, Washington, D. C.

**UNFERMENTED REDUCING SUBSTANCES IN MOLASSES:**

F. W. Zerban, Sugar Trade Laboratory, 113 Pearl St., New York City.

**DIACETYL:**

J. B. Wilson, Food and Drug Administration, Washington, D. C.

**DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:**

C. F. Snyder, National Bureau of Standards, Washington, D. C.

**HONEY AND HONEYDEW HONEY:**

G. P. Walton, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

**SUCROSE AND ASH IN MOLASSES:**

R. A. Osborn, Food and Drug Administration, Washington, D. C.

**REFRACTIVE INDICES OF SUGAR SOLUTIONS:**

C. F. Snyder

**WATERS, BRINE, AND SALT:**

*Referee:* A. E. Mix, Food and Drug Administration, Washington, D. C.

**FLUORINE AND BORON:**

A. E. Mix

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**MEMBERS AND VISITORS PRESENT, 1940 MEETING**

Acree, Fred, Jr., Bur. of Entomology & Plant Quarantine, Washington, D. C.  
Adams, J. R., Bur. of Plant Industry, Washington, D. C.  
Adams, J. Rivers, California Chemical Company, New York City.  
Adams, Waldo L., Agricultural Experiment Station, Kingston, R. I.  
Alfend, Samuel, Food and Drug Administration, St. Louis, Mo.  
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Allen, Raymond N., Gorton-Pew Fisheries Co., Gloucester, Mass.  
Allen, W. S., General Chemical Co., 40 Rector St., New York City.  
Allison, Andrew W., Food and Drug Administration, Buffalo, N. Y.  
Allison, Franklin E., Bur. of Plant Industry, Washington, D. C.  
Almy, L. H., H. J. Heins Co., Pittsburgh, Pa.  
Alter, Abraham, Food and Drug Administration, Baltimore, Md.  
Atwater, C. A., The Barrett Co., 40 Rector St., New York City.  
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Badertscher, A. Edison, McCormick & Co., Baltimore, Md.  
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Bailey, L. H., Bur. of Agricultural Chemistry & Engineering, Washington, D. C.  
Baird, Fuller D., National Oil Products Co., Harrison, N. J.  
Baker, Warren S., Chas. M. Cox Co., Boston, Mass.  
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Balls, A. K. Bur. of Agricultural Chemistry & Engineering, Washington, D. C.  
Barbella, Nicholas G., Bur. of Animal Industry, Washington, D. C.  
Barker, Donald K., The Borden Co., Elgin, Ill.  
Barthen, C. L., White Laboratories, Inc., Newark, N. J.  
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Bartram, M. Thomas, Food & Drug Administration, Washington, D. C.  
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Bates, Frederick, National Bureau of Standards, Washington, D. C.  
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Beyer, G. F., Bureau of Internal Revenue, Washington, D. C.  
Bides, P. R., State Chemical Laboratory, Auburn, Ala.  
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Bonney, V. B., Food and Drug Administration, Washington, D. C.  
Bopst, L. E., Associate State Chemist, College Park, Md.



- Bowen, C. V., Bur. of Entomology & Plant Quarantine, Washington, D. C.  
 Bowes, F. C., New England By-Products Corp., 177 Milk St., Boston, Mass.  
 Bowman, Walker.  
 Breed, Robert S., Agricultural Experiment Station, Geneva, N. Y.  
 Brewster, J. F., National Bureau of Standards, Washington, D. C.  
 Briesse, R. R., Bur. of Animal Industry, Washington, D. C.  
 Brinton, C. S., Food & Drug Administration, Philadelphia, Pa.  
 Brooke, Richard O., Wirthmore Laboratory, Malden, Mass.  
 Broughton, L. B., State Chemist, College Park, Md.  
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 Buchanan, P. J., 50 Church Street, New York City  
 Burton, T. H., Auburn, Ala.  
 Butt, C. A., International Agricultural Corp., East Point, Ga.
- Caldwell, Paul, Darling & Co., East St. Louis, Ill.  
 Callaway, Redman, Laboratory Construction Co., Kansas City, Mo.  
 Cannon, H. J., Laboratory of Vitamin Technology, 1411 E. 60th St., Chicago, Ill.  
 Capps, Hubert H., Department of Agriculture, Washington, D. C.  
 Carpenter, F. B., Va.-Carolina Chemical Corp., Richmond, Va.  
 Carroll, E. C., Jr., Gorton-Pew Fisheries Co., Gloucester, Mass.  
 Carson, C. T., Frankfort Distilleries, Inc., Baltimore, Md.  
 Carter, R. H., Bur. of Entomology & Plant Quarantine, Washington, D. C.  
 Casey, F. W., Food & Drug Administration, Washington, D. C.  
 Chapman, Fred M., Somerville, Mass.  
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 Clark, Kenneth G., Bur. of Plant Industry, Washington, D. C.  
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 Clarke, W. Tresper, Rockwood & Co., Brooklyn, N. Y.  
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 Concannon, C. C., Bur. of Foreign & Domestic Commerce, Washington, D. C.  
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Ellis, N. R., Bur. of Animal Industry, Beltsville, Md.  
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Evenson, O. L., Food and Drug Administration, Washington, D. C.

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Feinstein, Louis, Agricultural Marketing Service, Washington, D. C.  
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Fitzhugh, O. G., Food and Drug Administration, Washington, D. C.  
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Fritz, James C., Borden Company, Elgin, Ill.  
Fuerst, Robert C., University of Maryland, College Park, Md.  
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Gay, W. L., F. W. Berk & Co., Inc., New York City.

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Gephart, F. C., 23 E. 31st St., New York City.

Gerritz, Harold W., Food and Drug Administration, San Francisco, Calif.

Gilmore, L. N., Maritime Milling Co., Buffalo, N. Y.

Ginn, Wells W., Chemical Novelties Corp., Cincinnati, O.

Glycart, Chris K., Food and Drug Administration, Chicago, Ill.

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Haller, Herbert L. J., Bur. of Entomology & Plant Quarantine, Washington, D. C.

Halliday, George E., Bureau of Plant Industry, Washington, D. C.

Halvorson, H. A., Department of Agriculture, Dairy and Food, St. Paul, Minn.

Hammond, J. W., Agricultural Experiment Station, Knoxville, Tenn.

Hammond, Lester D., National Bureau of Standards, Washington, D. C.

Hanson, H. H., State Chemist, Dover, Del.

Hardesty, John O., Bureau of Plant Industry, Washington, D. C.

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Hartmann, B. G., Food and Drug Administration, Washington, D. C.

Hasbrouck, F. F., Allied Mills, Inc., Peoria, Ill.

Haskins, Arthur L., Pennsylvania State College, State College, Pa.

Haynes, Herman W., Food and Drug Administration, Boston, Mass.

Hays, William K., Food and Drug Administration, Washington, D. C.

Heath, Walter H., Food and Drug Administration, Boston, Mass.

Heiss, Louis R., American Instrument Co., Silver Spring, Md.

Helsel, W. G., Food and Drug Administration, Washington, D. C.  
Henry, Arthur M., Food and Drug Administration, Atlanta, Ga.  
Herd, R. L., Food and Drug Administration, Washington, D. C.  
Herman, A., Jos. E. Seagram & Sons, Louisville, Ky.  
Herrick, H. T., Bur. of Agricultural Chemistry & Engineering, Washington, D. C.  
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Hibben, James H., U. S. Tariff Commission, Washington, D. C.  
Hildebrandt, F. W., U. S. Industrial Chemicals, Inc., Curtis Bay, Baltimore, Md.  
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Hodges, F. A., Food and Drug Administration, Washington, D. C.  
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## PRESIDENT'S ADDRESS\*

### SOME RELATIONS OF ANALYTICAL SCIENCE TO THE WORK OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

By W. W. SKINNER (U. S. Bureau of Agricultural Chemistry and  
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It is a great honor to be chosen the President of the A.O.A.C. The honor carries with it certain definite responsibilities, however, and one of these is an appearance before you on this occasion for the discussion of some subject of interest and profit to the organization.

I have carefully reviewed the efforts of my distinguished predecessors at previous annual meetings of our Association, seeking inspiration to guide me at this time. Some of the other presidents have brought to you the fruits of their labor and their experiences, and these presentations in the aggregate make a Veda of great value. I decided that I might contribute an equally valuable part by departing from this time-honored style of Presidential address by presenting to you a brief review of the Association's financial and spiritual assets.

I would not speak of our financial condition at this time if all our members remained for the Wednesday session of our annual meeting and heard the report of the Treasurer, but I desire to get into the record a few remarks in the hope that you may be stimulated to examine critically the prepared report, which is supported by the Auditing Committee and the statement of the certified public accountant accompanying the report. Moreover, I hope you will find cause to bring to the Executive Committee suggestions for directing the future development of the Association.

The period 1915 to 1920 was the most critical in the financial history of the Association. The publication of *The Journal* had proved to be unprofitable, and there were no surplus funds in the treasury. A controversy with our printer had culminated in the filing of a lawsuit against the Association and some of its responsible and managing members living within the jurisdiction of the Court in the District of Columbia. In fact the Association was bankrupt.

A conference of interested persons was called, and a reorganization was determined upon and effected. As a result, the Association was incorporated under the laws of the District of Columbia, the suit of the printer was compromised and settled out of court, and the work of the Association was organized on a sectional basis, such as prevails today. New interest was stimulated in the publication of our standard book of methods and plans for its revision every five years. This decision was the most momentous since that day in September, 1884, when a little band of official

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chemists met in Philadelphia and organized the Association of Official Agricultural Chemists.

During the 20-year period, 1920-1940, *The Journal* has been vastly improved and developed to a self-sustaining position, and *Methods of Analysis* has been revised and issued as planned in 1925, 1930, 1935, and 1940. Each edition prior to 1940 (which is just off the press) has been disposed of to such advantage that the Association has found it possible to abolish all dues; to reduce the price of the *Book of Methods* to members from \$5 to \$4; to liquidate all of its old debts; to establish a satisfactory working balance; and to build up a reserve fund presumably sufficient to meet all of its legitimate needs. All of this is set forth in the dry figures of our annual financial reports, which I hope many of you will read.

But it is not the material assets of the Association that I particularly wish to discuss at this time, but rather its spiritual assets, those tenets that were formulated by the founders of this organization, and upon which we have built and are continuing to build this structure. In 1884 that little band of pioneers—only one of whom, Dr. Charles W. Dabney, is now living—set forth the objectives of this brotherhood, which were later formulated as an article of our constitution as follows:

- (1) to secure uniformity and accuracy of methods, results and modes of statement of analysis of fertilizers, soils, cattle food, dairy products and other materials connected with agricultural industry; (2) to afford opportunity for a discussion of matters of interest to agricultural chemists.

In his Presidential address of 1889, after five years of activity by the Association, Dr. John Meyers recapitulated the objectives of the Association as follows:

It is aiming to lay a foundation so solid that every court in this land must respect its conclusions, and every analytical chemist, whether he lives in this country or elsewhere, must be forced either to practice or admit the advantages and correctness of our system of analyses. Step by step we have advanced through the difficult problems before us, feeling that we have steadily gained ground and that we are molding public sentiment in a manner that must redound to the advantage of the whole country and secure the highest respect for the science to which we are devoting our lives.

On the basis of those precepts there was outlined by the pioneers a definite plan of action. That original plan has developed into our unique system of research on methods of analysis, which is now the bulwark of our professional standing. This system has been perfected and preserved—but not without some criticism and dissent—dissension that has in some cases led to the separation of groups originally associated with us, or has stimulated the organization of new groups whose aims could not be reconciled or accommodated within the horizon of this Association. I refer to such organizations as the American Leather Chemists Society, the American Oil Chemists Society, the American Association of Cereal Chemists, and the

newest of the brotherhood, the Institute of Food Technologists. These developments are neither regretted nor deplored. They serve functions collateral to those of this Association of official analysts, which is devoted solely to public service. With the development of collateral groups from within our midst and with respect to their aims, which may seem to parallel our aims, we can fully sympathize. But in my opinion we should adhere with all tenacity to the original course plotted for this Association. That course was planned by the founders—thoughtfully and unselfishly—and I have heard no convincing argument for departing from it.

The method of mass research in the perfection of the tools of our profession is essentially a conservative method—the fusing together of inspiration and practice. It is out of such fusion here and elsewhere that experience is born. Conservatism is the bulwark of our position. But I must remind you that conservatism is not static. The radicalism of yesterday is the liberal conservatism of today, and the reaction of tomorrow. My idea of a proper definition of a desirable and safe conservatism is that it is a goodly portion of liberalism fused with just enough reaction to produce a spiritual alloy best suited to the immediate need. I am aware of past criticism and of the criticism that exists right now of our deliberate, and what appears at times to be over-cautious, consideration of changes and innovations in the mode of development and acceptance of our methods of analysis. Of this criticism we should now, as in the past, take careful note and pay due heed if the criticism is relevant and just. But we must not set our mental sails so as to have them buffeted unduly by the enthusiastic whirlwinds of the new and novel. Neither may we safely permit to go unchecked a tendency toward mental sclerosis—that hardening of the mind that indicates senility and decay.

Let me briefly examine the record to determine whether or not the charge of ultra-conservatism has merit. I have here before me, as exhibit No. 1, the first *Book of Methods* of this Association, a publication of some 80 pages, issued in 1899, and here as exhibit No. 2, the *Book of Methods* of 1940, a volume of 757 pages. From a quantitative basis, at least, these exhibits refute the charge of ultra-conservatism. And what about the record as evidenced by the individual methods? In reviewing material during the preparation of this paper I was amazed and not a little startled at the changes that have occurred in some of our standard or official methods. Take, for example, one of our first official methods, that for the determination of nitrogen. Many of the working chemists in this audience may not know that it was the old Dumas cupric oxide method, in which the actually evolved volume of gaseous nitrogen was collected, measured, and reduced to standard conditions in order to arrive at the true percentage. I imagine the worker in a modern fertilizer or feed control laboratory today, with the urgent demand for economical, rapid, and reliable analytical procedures, would be aghast at the necessity of using such a method.

And perhaps very few of you have ever made a determination of the nitrogen in a fertilizer, a food, or a feed by another old official method, the Ruffle method. Neither of these methods has survived as official for the work of this Association, not because of any lack of accuracy, but because they are too arduous and time-consuming.

In the development of the Kjeldahl, Gunning, and other wet combustion methods for the determination of nitrogen with a high degree of precision, accuracy, and speed, this organization has played the leading part. Of the changes in the early methods for the determination of phosphoric acid, I have most distinct recollections. Because this is more of a personal message than a professional address, I think I am permitted to make a personal reference to my first contact, in the fall of 1894, with the work of this Association. I well remember with what vigor the conservatives of that day discussed the rank heresy of the proposed adoption of a volumetric method for the determination of phosphoric acid—a method that had been developed shortly before by Pemberton and modified by Kilgore, a former President of this Association. Look in your copy of the 1940 methods and see what is available to you today. I might go on and offer in rebuttal to the charge of over-conservatism the change and development in methods for soils, insecticides, tanning materials, drugs, feeds, and food recorded in the 53 chapters of our *Methods of Analysis, A.O.A.C.*, but it would weary you. I have accomplished my purpose if I have even in some small degree stimulated or revived your interest in the philosophy of this Association.

One sage has expressed a human desire "to see ourselves as others see us." Fortunately we may do this in part by noting our reflection. One of our contemporaries has said of our methods:

The descriptions of procedure are very carefully edited and intended to allow of no ambiguity of detail for the exact performance of the determinations. Such thoroughness has resulted in a work unique in the literature of analytical chemistry.

And from a London journal:

It would be difficult to find any book in the range of analytical chemistry that is quite like the present edition, the result of a large amount of carefully planned and executed collaborative research work.

And from a South African reviewer:

The work amply fulfils its claims and covers the requirements of chemists engaged in the various ramifications of agricultural chemistry.

And on our 40th birthday in 1924, in summing up the accomplishments of this Association, in which he had played a stellar role, Dr. Harvey W. Wiley said, "The most valuable contribution made to agriculture in the last 40 years has been that of the standardization of the chemical and physical methods of research in agriculture by this Association."

What then are some of the opportunities visible now upon which to plan the future work of the Association?

From what has just been said I think you will agree with me that chemical analysis has progressed a long way since the beginning of our Association. Many of the important questions of agricultural chemistry have been answered, not merely in terms of structural formulae whose acceptance is a matter of convention rather than an actuality, but answered in terms of practical significance to the man that grows food and to the man that eats it. Photosynthesis, respiration, and the utilization of fertilizer by plants are not theories but facts.

Our Association must continue its contributions to the analysis of agricultural products. In effect, this means the use of analysis as a means of assay. But we should not forget that progress in agricultural chemistry is also dependent upon another and equally important use of chemical analysis, namely, the identification of compounds. This is a branch of endeavor in which we have every right to engage assiduously. The chemist has also recently come into possession of a number of new analytical tools that surpass in their sharpness anything he ever dreamed of before.

An era of physical methods in chemical analysis seems to be getting well under way. To appreciate this, look at a modern pH meter, or at a photoelectric colorimeter. Since when has it been possible to determine the hydrogen-ion concentration in less than a minute and write down the pH value of two decimals? How long have chemists been able to make accurate determinations of phosphorus on a few milligrams of sample?

It must be remembered that the interesting new substances of today, such as the hormones, the enzymes, and the vitamins, usually occur in traces. A ton of starting material may yield only a few grams of the sought-for substance. By making full use of the modern methods of micro-analysis, the chemist finds that even a very small quantity of material is often sufficient for a complete identification; 10 mg. will do for the micro combustion to determine carbon and hydrogen; only 2 or 3 mg. is needed for nitrogen in the micro Kjeldahl apparatus, which gives the answer in 30 minutes; and perhaps 1 mg. is ample for a phosphorus determination. I wish the same could be said for sulfur, but this determination is one of the most extravagant, and uses perhaps 25-50 mg. of sample.

We could go from one new micro method to another until dusk—each of them valuable for one purpose or another—for determining methoxyl groups or free amino groups or carboxyl groups and so forth. Each determination fixes some detail of the hitherto unknown molecule, or offers a new possibility for getting information about the constitution of a new substance.

A theme that is most pressing just now is the development of methods necessary for the determination of the trace elements in mineral, plant, and animal material. The great interest of chemists and others in the sig-

nificance of such traces in soils, fertilizers and plant products has brought serious problems of analytical technic. Some of the older methods that have been quite adequate for our needs may have to be revised to meet this new situation. Or, in many cases it may be necessary to develop entirely new chemical, physical, or biological procedures. Our workers and our referees need to give more attention to the development of micro methods, a fruitful field of research. We have as you know a section and a referee on this subject, but interest needs to be stimulated if the position of leadership that we have attained in our chosen field is to be maintained.

The analyst, however, is faced with other difficulties besides that of having to be satisfied with tiny supplies of material. One of the greatest of these is the surety that what he investigates is a single substance and not a haphazard mixture. In many cases before the actual analysis must come the labor of purification, and with it the often embarrassing question of when the purification is sufficiently complete. Modern methods of purification are perhaps of equal importance with modern methods of analysis. Crystallization is still an accepted method of achieving purity. Not that crystals are necessarily pure substances, but usually they are, and always they are reproducible material. This is why crystalline enzymes are valuable in agricultural research.

The proof of purity of crystals has required extensive study in recent times, especially the purity of protein crystals. Some criterion of purity is needed and a determination of the solubility seems to be the answer. Pure crystals have a constant solubility in their own solutions; mixed crystals have not. This single fact has made great advances possible in protein chemistry and particularly in the chemistry of the enzyme proteins.

With more critical tests for purity has come the development of easier methods for purification. In the last century gold and copper were purified by electrolysis. The same principle has recently been applied in the separation of the proteins in blood serum. In our Bureau of Chemistry and Engineering, M. J. Horn has purified his selenium compound from wheat in an electrophoresis apparatus at a pressure of several thousand volts.

Another modern tool, distinctly of the high pressure variety, is the ultracentrifuge. At 50,000 times gravity (an easy attainment with a modern ultracentrifuge) a gram weight exerts a pressure of about 110 pounds. Naturally a large particle begins to feel its relative importance under such conditions (and proteins are composed of large particles). In the ultracentrifuge the molecules of different proteins sediment at different speeds, depending upon their weights and shapes. It is frequently possible thus to separate proteins from each other completely enough to allow of their later crystallization in practically pure form. The analyst feels no pain in such an operation (which might otherwise require weeks of work), once he has constructed the machine. On the other hand, that construction is



attended by a peculiar physiological disturbance known as a headache.

Once separated in pure form the material is not only ready for analysis but also for the many physical measurements by which the analytical results may be interpreted. If the centrifuge has not given some idea of the molecular weight, osmotic pressure measurements may give the answer. To the modern chemist it is a real pleasure to make dialysis with a membrane that he knows isn't going to break whenever the osmotic pressure is high enough to be interesting.

The hidden intricacies of the molecule are often of more interest than its size, and some of these become visible in the spectroscope. A benzene ring or any other configuration that contains a vibrating bond must absorb light at some particular wave length, and the absorption spectrum may inform us as to what that wave length is. Because different configurations absorb at different wave lengths, accurate knowledge of some of the constituents of a molecule can often be obtained. It is hard to see how the constitution of the hemins, the cytochromes, or the respiration enzyme could have been worked out, otherwise. A good spectroscope is so delicate that not milligrams of substance, but gamma (millionths of a gram) are all that the analyst requires. Thus the centrifuge and the spectroscope have become associates of the polariscope, the microscope, the refractometer, and the balance as essential working tools of the chemist, and he now talks glibly of Ångström units and micrograms as the patriarchs did of milligrams and cubic centimeters.

The importance of this and much more that could be said along the same line is difficult to overestimate. You are in a position to realize it, but it is a great pity that the public cannot be made to realize it also. A few milligrams of crystals may hold the secret of a great principle of agriculture. In agricultural materials, they constitute some product of that mysterious thing we call life. Their identification may unlock mysteries of growth and maturity; of health and disease; of life and death. The methods that lie at our hand permit us now to investigate such things, to make use of what is benign, and to discard what is harmful. The urge to study them is tremendous, and the knowledge that derives therefrom is power over the future, to direct and shape our destiny.

## ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

## THIRD DAY

### WEDNESDAY—AFTERNOON SESSION

#### REPORT OF EDITORIAL BOARD

By W. W. SKINNER, *Chairman*

Some of the facts you already know. The *Book of Methods* has been issued, and you have had the pleasure of seeing it. It was decided by the Executive Committee that 7,500 copies of this edition should be ordered (5,000 bound), because the anticipated demand seemed to justify it.

Perhaps you might be interested in knowing that when it became necessary to make the first payment on the *Book of Methods*, we approached our banker and offered as collateral \$5,500 in government bonds, but the cashier pointed out there would be some difficulty because of the restrictions placed upon registered bonds by the U. S. Treasury Department. He finally said, "Give me a ninety-day note and put your bonds back in the safety deposit box." This was rather a thrill, because it was evidence that the credit of the Association is pretty sound. We then borrowed the money on the note of the Association, signed by Mr. Lepper and me as authorized by the Executive Committee.

I desire at this time to record a vote of appreciation in behalf of the Association to the members of the Revision Committee, and especially to E. M. Bailey, Chairman, and his assistant, H. J. Fisher. The work was unusually heavy and taxing, as it was done in conjunction with their own important duties, but their interest was sustained throughout the ordeal.

I shall now ask Dr. Bailey to make a detailed report about the issuing of the *Book of Methods*.

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#### REPORT OF COMMITTEE ON REVISION OF *METHODS OF ANALYSIS*

The fifth edition of *Methods of Analysis* is completed, and it is already in your hands. The book itself constitutes our report; and the preface written by our President, Dr. Skinner, leaves little to be added here.

About 1,000 copies have been distributed. Very few errors have been reported, but it is too much to hope that others will not be found. It is urgent

that referees and others interested read the chapters carefully and report any corrections that should be made in order that a list of such may be compiled for distribution as soon as possible.

Since the first edition twenty years ago our book has nearly doubled in volume of subject matter. The first edition contained 417 pages; the present one has 757 pages. The increments for the several 5-year periods have not been uniform; they have varied from 47 pages to 118 pages and average 70 pages. The present edition contains only 47 pages more than the fourth edition. This small increase is for the most part due to space-saving devices introduced in the third edition and carried further in this one.

There is, of course, a constant demand for new and improved methods in all the fields covered by our official text. At the moment this is particularly true in the field of foods, drugs, and cosmetics, due to recent legislation, which results in attaching a new significance and importance to our methods. For example, food definitions and standards which, under the new act, now have legal force and effect, predicate numerical standards and limits of composition on methods of procedure specifically cited in the official text of this Association.

Material has now been supplied for four chapters that in the fourth edition were designated without text; and our collaborative program has been enlarged to meet the need for methods in some of the newer fields of interest.

Your committee does not need to remind you of the debt we owe to our referees, associate referees, and collaborators, not only for their help in preparing this revision, but for the thought and study that they give each year to all the subject matter of our text. As you well know, this book, and all its predecessors, represent their work.

We submit this fifth edition and move its adoption.

E. M. BAILEY, *Chairman*

L. E. WARREN

J. W. SALE

G. G. FRARY

H. A. LEPPER

MARIAN E. LAPP

Approved.

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#### REPORT OF THE EDITORIAL COMMITTEE OF *THE JOURNAL*

By HENRY A. LEPPER, *Editor and Chairman*

Volume XXIII of our *Journal*, published during 1940, contains 844 pages, the largest volume in the history of its publication. Previously, Volume VIII, which consists of 6 numbers issued over a period of a year and a half, had stood out with its 744 pages as the goal to be passed. How-

ever, it is not size alone that counts; it is the diversity of subject matter and the scientific tone and quality of the reports and papers making up these many pages that bring to us a justifiable feeling of pride, because of the realization that a valuable contribution is thereby made to the permanent records of the fields in which we have chosen to work.

It is gratifying that with an increased output *The Journal* has returned a profit, as shown by the report of the treasurer.

Foreign subscriptions have declined owing to the unsettled conditions abroad but the total number of subscriptions is in excess of last year's. We have had several requests from China for free or half-rate subscriptions. We believe that this shows that our *Journal* fulfills a definite need among fellow workers the world over. It also indicates that similar requests may be anticipated from other war-torn countries if and when peace returns. The Executive Committee has authorized the Editorial Board to meet such requests.

In 1930, we published an index of the proceedings and publications of the Association from its beginning up to and including 1929. An index for the decade 1930-1939 has been authorized and will appear during the coming year.

The granting of 50 reprints, which previously had been intended for authors of contributed papers only, has been extended to referees whose reports represent original work such as would constitute contributed papers. It has frequently been the policy in publishing referee reports to omit the details of a method if they are published in the report on Changes of Methods by reason of the adoption of the method, and to include it by reference only. This policy was one directed at conservation of space and funds. Comment has been offered that such presentation makes for inconvenience in reading the report and also makes the report incomplete as a reprint. The editorial office has tried to obviate the latter difficulty by not deleting the directions for methods where the requests of referees for reprints have been granted. This has not always worked out successfully, and with the next volume a policy of printing more complete reprints has been approved.

No report of this editor would be complete without a statement urging our members to avail themselves of the opportunity to use the pages of their *Journal* as an established medium for presentation of the results of their original investigations. They should take advantage of the recognized standing of our *Journal* as foremost among purely scientific publications and at the same time contribute to the retention of such prestige.

Approved.

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No report was given by the Committee on Quartz Plate Standardization and Normal Weight.

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REPORT OF COMMITTEE ON STANDARDIZATION  
OF GLASSWARE

*Majority report.*—The following is the report of the majority of the committee appointed to study the policy of adopting methods for testing the accuracy of graduated glassware and other apparatus.

Accurate analytical work requires accurate measuring instruments as well as accurate methods. Errors may occur in graduated glassware, weights, or other measuring instruments as purchased, or they may develop during use. Since it is the primary object of the A.O.A.C. to secure accurate analytical results, the majority of the Committee recommends the policy of adopting methods for testing and checking the accuracy of volumetric flasks, burets, and other measuring instruments, in full cooperation with the Bureau of Standards. These methods are to be used on instruments not already tested by the Bureau of Standards, or for testing such instruments as may have deteriorated through use.

One member of the committee (Mr. Lythgoe) does not agree with the majority and has been requested to turn in a minority report. This important matter should, of course, have full consideration from all points of view.

G. S. FRAPS, *Chairman*  
W. F. HAND

*Minority report.*—I am opposed to placing in the official methods of the A.O.A.C. details as to methods of calibrating weights and measures. It is to be assumed that any person who calls himself a chemist knows the necessity of using accurate weights and measures in his work, and, furthermore, he should have been properly instructed as to the methods of assuring himself of such accuracy. The standard works on chemical analysis contain methods for such calibration. They can be found in Fresenius, in Treadwell-Hall's *Quantitative Analysis*, and in many of the chemical handbooks. Directions may also be found in the *U. S. Pharmacopoeia*. If methods of this character are placed in the official methods of the Association, it will be a decided disadvantage in the case of litigation if the attorney opposed to the chemist making the analysis requires proof that the chemist himself actually calibrated the weights and measures he used.

HERMANN C. LYTGOE

These reports were accepted by the Association, and the subject was retained for further consideration.

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## REPORT OF COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS

### *Official, Final Action*

#### CALCIUM NITRATE

*Calcium nitrate* (nitrate of lime) is a commercial product consisting chiefly of calcium nitrate, and shall contain not less than fifteen per cent (15%) of nitrogen.

#### AMMONIATED SUPERPHOSPHATE

*Ammoniated superphosphate* is the product obtained when superphosphate is treated with ammonia or with a solution containing free ammonia and other forms of nitrogen dissolved therein.

#### SUPERPHOSPHATE

*Superphosphate* is a product obtained by mixing rock phosphate with either sulfuric acid or phosphoric acid or with both acids. (The grade that shows the available phosphoric acid shall be used as a prefix to the name. *Example*: 20 per cent superphosphate.)

The definition of superphosphate on page 734 of *Methods of Analysis, A.O.A.C.*, 1940, shall be dropped and the above definition substituted.

### *Second Reading as Tentative*

#### NITRATE OF SODA AND POTASH

*Nitrate of soda and potash* is a commercial product containing nitrates of sodium and potassium and it shall contain not less than fourteen per cent (14%) of nitrogen (N) and fourteen per cent (14%) of potash ( $K_2O$ ).

#### GUARANTEEING IN TERMS OF ELEMENTS

All fertilizer components with the exception of potash ( $K_2O$ ) and phosphoric acid ( $P_2O_5$ ) if guaranteed shall be stated in terms of the elements.

### *Proposed Definition*

#### MAGNESIUM OXIDE

*Magnesium oxide* is a commercial product containing not less than ninety-two per cent (92%) of the oxide of magnesium.

Approved.

L. S. WALKER, *Chairman*

W. C. JONES

G. S. FRAPS

L. E. BOPST

W. H. MACINTIRE

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## REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

By HENRY A. LEPPER, *Chairman*

The work of this committee continues apparently unabated. It is made difficult by the failure of many referees to appreciate the necessity for the submission of reports well in advance of the meeting. Each year the existence of several reports is learned only when they are presented at the

designated time on the program. The recommendations of this committee are of fundamental importance in the furtherance of the Association's program of work, and too much appreciation cannot be extended to the members of the four subcommittees who labor long and diligently, foregoing attendance at the sessions, so that the Association may be advised of appropriate action to be taken in the adoption and changes of methods. The many referees are again requested to plan and execute the work to be undertaken for the next year so as to permit the preparation and submission of reports at the time designated in advance of the meeting. Such cooperation with your committee will make for greater efficiency in the consideration of recommendations and will afford greater opportunity for the committee members to meet other members and visitors and to attend the various sessions, which all members should enjoy.

Approved.

## REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES\*

By H. H. HANSON (*Acting Chairman* for G. E. GRATTAN, who was unable to be present), H. A. HALVORSON, and E. L. GRIFFIN

### STANDARD SOLUTIONS

It is recommended—

(1) That the method for the standardization of potassium permanganate (p. 653, 14, 15) be adopted as official (final action).

(2) That the preparation of standard hydrochloric acid solutions from constant boiling acid be studied.

(3) That the preparation and standardization of sodium thiosulfate solutions be studied.

(4) That the method for the standardization of sulfuric acid solutions by means of specific gravity (p. 652, 13) be adopted as official (first action).

(5) That the method for the preparation of standard arsenous oxide solution recommended by the associate referee be adopted as official (first action).

(6) That the method for the preparation and standardization of iodine solutions recommended by the associate referee, with the inclusion of the supplemental precautions suggested by the referee, be adopted as official (first action).

(7) That the Mohr and Volhard methods submitted by the associate referee for the standardization of silver nitrate solutions be adopted as official (first action).

(8) That direct methods for the standardization of thiocyanate solutions be studied.

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\* These recommendations, submitted by Subcommittee A, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis*, A.O.A.C., 1940.

## INSECTICIDES AND FUNGICIDES

It is recommended—

(1) That the precautions submitted by the Associate Referee on Fluorine Compounds be included in the lead chlorofluoride method for fluorine (p. 49, 19).

(2) That collaborative study of methods for the determination of total fluorine be conducted next year.

(3) That collaborative study of a method for the determination of silicofluoride in admixture with sodium fluoride be conducted next year.

(4) That the mercury reduction method for the determination of Pyrethrin I in pyrethrum powder (p. 66, 112–113) be adopted as official (final action).

(5) That the crystallization method for the determination of rotenone in derris and cube powder (p. 64, 110) be adopted as official (final action).

(6) That the method for total ether extract in derris and cube powder (p. 66, 111) be adopted as official (final action).

(7) That studies on naphthalene in poultry lice products be continued.

## FEEDING STUFFS

It is recommended—

(1) That an associate referee be appointed to study the application of the acid hydrolysis method for fat (p. 213, 11) to fat in cooked animal feeds containing cereals.

(2) That an associate referee be appointed to study a 4-hour ether extraction procedure for the determination of crude fat or ether extract in stock feeds.

(3) That an associate referee be appointed to study the use of filtration aids for the crude fiber determination.

(4) That the precautionary notes suggested by the referee be added to the method for the determination of grit in poultry and similar feeds (p. 365, 46).

(5) That an associate referee be appointed to study the determination of salt in feeding stuffs.

(6) That no further attention be given to castor seed at present.

(7) That the method for the determination of ammoniacal and urea nitrogen recommended by the referee be adopted as tentative, and that an associate referee be appointed to study the determination of urea and inorganic nitrogen salts in stock feeds.

(8) That study of the method for the determination of ash in stock feeds be continued.

(9) That study of the methods for the determination of calcium and iodine in mineral mixed feeds be continued.

(10) That the work on lactose be continued along the lines outlined by the associate referee.

(11) That the study of methods for the determination of fat in fish meal be continued.



(12) That the heading "Carotene-Tentative" (p. 369, 61) be changed to read "Crude Carotene in Hays and Dried Plants—Tentative."

(13) That the use of a photoelectric colorimeter in the determination of carotene described by the associate referee be adopted as tentative.

(14) That the study of the methods for the determination of crude and pure carotene be continued.

(15) That the determination of carotene and cryptoxanthin in yellow corn be studied.

(16) That the study of methods for the determination of the adulterants of condensed milk products and cod-liver oil be continued.

(17) That the method for the determination of manganese (p. 369, 59-60) be changed as suggested by the associate referee, and that the method be entitled "Acid-soluble Manganese in Grain and Stock Feeds" and adopted as official (first action).

(18) That the study of manganese be continued.

(19) That study on devising standard methods of sampling be continued.

#### FERTILIZERS

It is recommended—

(1) That the Associate Referee on Phosphoric Acid continue the study of the methods for moisture.

(2) That the ferric sulfate-dipotassium phosphate method for shortening the Kjeldahl digestion be studied by the Associate Referee on Nitrogen.

(3) That the beaker method for the determination of water-insoluble nitrogen (35, p. 29) be adopted as official (final action).

(4) That study of the four methods for platinum recovery published in *This Journal*, 22, pp. 286-7, or other methods be continued.

(5) That collaborative work on a number of samples be done to determine the effect of temperature on the solubility of potassium chloroplatinite in acid-alcohol and alcohols in the regular potash determination.

(6) That the words, "If not, dissolve the  $K_2PtCl_6$  in hot  $H_2O$ , reweigh, and make correction for water-insoluble residue," be included in the parentheses of the last line of par. 42(a), p. 31.

(7) That the study of elimination of water-insoluble material coarser than 20 mesh be continued by the Associate Referee on Acid- and Base-forming Quality.

(8) That the basicity of phosphate rock and other factors that affect the method be studied further by the same associate referee.

(9) That fertilizer materials and moist fertilizer mixtures be ground for analysis to pass a sieve with 1 mm. circular openings or a 20-mesh Tyler standard sieve, and that dry mixtures that show a tendency to segregate may be ground to pass a 35-mesh Tyler standard sieve.

(10) That Methods 1, 3, and 4 for the determination of acid-soluble calcium given in the report of the associate referee be further studied collaboratively.

(11) That Method 1 for the determination of acid-soluble calcium in mixed fertilizers be adopted as tentative after correction as suggested by the associate referee.

(12) That the tentative method for the determination of acid-soluble calcium (47, p. 34) be changed as suggested by the associate referee and remain tentative.

(13) That methods for the determination of sulfur in mixed fertilizers be studied.

(14) That the volumetric method for the determination of copper, adopted as tentative last year (p. 34, 50-51), be revised as suggested by the associate referee and remain tentative.

(15) That the colorimetric method for the determination of copper described in the associate referee's report be further studied.

(16) That the gravimetric method for the determination of zinc described in the associate referee's report be adopted as tentative for samples containing more than 0.20 per cent of zinc.

(17) That the volumetric method for the determination of zinc described in the associate referee's report be further studied.

(18) That the dithizone method for the determination of zinc, proposed by the associate referee for plants, be studied collaboratively for the determination of zinc in fertilizers.

(19) That the method for the determination of magnesium in water-soluble compounds (55, p. 36) be adopted as official (final action).

(20) That Method II for the determination of acid-soluble magnesium (p. 36, 53; *This Journal*, 22, 270; 23, 247) be revised as suggested by the associate referee, and then adopted as official (first action).

(21) That Method III, volumetric modification of the method for the determination of acid-soluble magnesium (p. 36, 54), be adopted as official (first action).

(22) That the study of methods for the determination of acid-soluble, water-soluble, and active magnesia in fertilizers be continued.

(23) That the colorimetric method for the determination of acid-soluble manganese (*This Journal*, 23, 250) be adopted as official (first action), and that it be entitled "Applicable to samples with not more than 5% manganese."

(24) That the bismuthate method for the determination of acid-soluble manganese described as Method 5 in the associate referee's report be adopted as tentative.

(25) That the study of methods for acid-soluble manganese, including the potassium periodate volumetric method, be continued.

**SOILS AND LIMING MATERIALS**

It is recommended—

- (1) That the technic of the distillation method for the determination of fluorine described by the referee be subjected to collaborative study.
- (2) That the work of the Associate Referee on Hydrogen-ion Concentration of Soils of Arid and Semi-arid Regions be continued.
- (3) That the work of the Associate Referee on Hydrogen-ion Concentration of Soils of Humid Regions be discontinued.
- (4) That further work be done on the extraction of exchangeable calcium and magnesium in the presence of carbonates.
- (5) That studies on the direct determination of exchangeable hydrogen in soils be continued.
- (6) That the work on boron be continued.

**PLANTS**

It is recommended—

- (1) That studies on iodine be continued.
- (2) That collaborative studies on the boron method described by the associate referee be initiated.
- (3) That the work on carbohydrates be continued.
- (4) That the method presented by the associate referee for the determination of zinc in plants, with modifications, be adopted as tentative, and that this method be subjected to further study with particular attention given to the ashing of the sample and extraction of the zinc from the plant ash.
- (5) That the Associate Referee on Zinc be authorized to continue a study of a method for the determination of iron in plants and initiate collaborative work on it.
- (6) That the studies on copper and cobalt be continued.
- (7) That the study of methods for the determination of carotene and chlorophyl in plant tissue be continued.
- (8) That the Petering-Wolman-Hibbard procedure be given additional study as a combined method for determining total chlorophyl and carotene, and that increased attention be given to the evaluation of chlorophyl.
- (9) That a study be made to determine the best methods for sampling fresh plant tissues and for handling samples of such materials (including use of machine grinding) prior to and during extraction of pigments therefrom.
- (10) That studies on hydrocyanic acid be continued.

**ENZYMES**

It is recommended that study of methods for the determination of papain be continued.

# LIGNIN

It is recommended that the study of methods for the determination of lignin in plants be discontinued for the present.

## PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

It is recommended—

- (1) That further studies be made on the procedure for skinning test, alkali resistance test, and soap resistance test.
- (2) That study of the procedure for determining elasticity or toughness of varnish films be continued.
- (3) That study of the methods of testing abrasion resistance and hardness of varnish films be continued.

## VITAMINS

It is recommended—

- (1) That study of the method for vitamin A be continued.
- (2) That the method for the determination of vitamin B<sub>1</sub> proposed by the associate referee be adopted as a tentative method.
- (3) That collaborative studies be conducted on the method for determining vitamin B<sub>1</sub>.
- (4) That the associate referee be requested to report on chemical methods for the determination of vitamin B<sub>1</sub> in flour and other cereal products at the next meeting.
- (5) That a uniform method for expressing degrees of heating for vitamin D milk be studied.
- (6) That the method for the determination of vitamin K proposed by the associate referee be adopted tentatively.
- (7) That collaborative studies be conducted on the method for the determination of vitamin K mentioned in Recommendation 6.
- (8) That the associate referee and referee be instructed to redraft the text of the method for vitamin K so that it will conform to the style used in *Methods of Analysis*, A.O.A.C., 1940.
- (9) That both the bacteriological and fluorometric methods for the determination of riboflavin described by the associate referee be tentatively adopted for riboflavin in yeast and dried skim milk.
- (10) That studies on the bacteriological and fluorometric methods for the determination of riboflavin be extended to liver meal, fish meal, alfalfa meal, and other materials.
- (11) That a primary standard for riboflavin be studied in order to have a method for checking the purity of riboflavin solutions used as standards.
- (12) That in par. 67, p. 372, the words "all chicks that weigh 100 grams or less and" be deleted from the text of the tentative method for vitamin D for poultry.

## REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES\*

By H. J. FISHER (Agricultural Experiment Station, New Haven,  
Conn.), *Chairman*; A. E. PAUL, and W. F. REINDOLLAR

### NAVAL STORES

No report was received. It is recommended that the subject be continued.

### RADIOACTIVITY

The referee recommends that the investigation of gamma ray measurements by means of the quantum counter be continued, and that beta ray measurements be studied. The Committee concurs in these recommendations.

### COSMETICS

The referee reports that owing to the broadness of the fields assigned to the associate referees, each one submitting a report confined his attention to one particular aspect of his field, as follows:

<i>Subject assigned</i>	<i>Topic studied</i>
Hair preparations	Coal-tar hair dyes
Colored make-up preparations	Lip sticks
Facial preparations	Cold creams

The referee recommends that the study of these topics be continued, and that more associate referees be appointed to study the following topics: Depilatories, deodorants, powders, sun preparations, and shampoos.

The Committee concurs in the recommendations.

### HAIR PREPARATIONS

The associate referee reports on methods for the extraction, separation, and identification of amino compounds used in hair dyes. He recommends that methods for the determination of *p*-phenylenediamine be studied collaboratively. The Committee concurs.

### COLORED MAKE-UP PREPARATIONS

The associate referee gives a list of preparations now on the market that come under this classification, and describes methods that have been used in the analysis of lipsticks. He recommends that the study of lip make-up preparations be continued, and that additional associate referees be appointed to study the following topics: Nail make-up, eye make-up, and face make-up.

The Committee concurs in these recommendations.

### FACIAL PREPARATIONS

The associate referee confined his attention principally to cold cream.

\* These recommendations, submitted by Subcommittee B, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis*, A.O.A.C., 1940.

He discusses methods for the separation of the common components of cold creams and recommends that the study of this topic be continued. The Committee approves.

DENTIFRICES AND MOUTH WASHES  
MISCELLANEOUS COSMETICS

No reports were received. It is recommended that these topics be continued.

COLORING MATTERS IN DRUGS AND COSMETICS

The referee recommends that the work of the associate referees be continued. The Committee concurs in this recommendation. It is the belief of the Committee, however, that the study of coloring matters in foods, drugs, and cosmetics should be placed under the supervision of one general referee on coloring matters. The products studied and the methods used are similar and in many cases identical. For these topics to be under the supervision of two different referees leads to duplication of work and might result in the adoption of inconsistent recommendations.

The Committee accordingly recommends to the Association that the separate refereeships on Coloring Matters in Foods and on Coloring Matters in Drugs and Cosmetics be combined as one general refereeship on Coloring Matters.

NON-PIGMENT COLORS

The associate referee submitted to collaborative study a modification of the titanium trichloride titration of dyes. He recommends further study. The Committee concurs.

LAKES AND PIGMENTS

The associate referee discusses the methods that have been used in determining the dye content of pigment colors. He recommends further study. The Committee approves.

ANALYSIS OF COLOR MIXTURES

The associate referee discusses methods used in the determination of mixed dyes, and recommends that further experimental work be carried out before collaborative study is attempted. The Committee concurs.

TESTS FOR COAL-TAR COLOR INTERMEDIATES

The associate referee describes a method for the determination of *p*-nitraniline. He recommends that the work on intermediates be continued. The Committee concurs.

SPECTROPHOTOMETRIC COLOR TESTING

The associate referee describes the uses of the spectrophotometer in the identification and determination of dyes and in the detection of impurities therein. He recommends that work on spectrophotometric color testing be continued. The Committee approves.

## MICRO METHODS FOR COAL-TAR COLOR ANALYSIS

No report was received. It is recommended that the subject be continued.

## SYNTHETIC DRUGS

The referee makes recommendations in regard to the work of certain of the associate referees. These will be commented on under the appropriate topics below. He also recommends that the following new topics be studied: Metrazol and sulfathiazol.

The Committee concurs in these recommendations.

## BENZEDRINE (RACEMIC DESOXY-NOR-EPHEDRINE)

The associate referee has developed a method for the determination of this substance which he recommends for collaborative study. The referee and the Committee concur in this recommendation.

## HYDROXYQUINOLINE SULFATE (CHINOSOL)

No report was received. It is recommended that this topic be reassigned.

## METHYLENE BLUE

This topic was assigned last year for the purpose of studying the possibility of combining the A.O.A.C. method of separation with the perchlorate method of determination. The associate referee did not study the perchlorate method, but did investigate the A.O.A.C. iodine volumetric method. He recommends a change in the factor for converting iodine to methylene blue, and also recommends the adoption of a method for the determination of moisture in methylene blue. The Committee does not think that an empirical factor should be adopted without collaborative study, and recommends that the topic be further studied with particular attention given to the recommendations in the Committee's report for 1939 (*This Journal*, 23, 59).

## AMINOPYRINE, ACETOPHENETIDIN, AND CAFFEINE

The associate referee submitted to collaborative study a method for the separation and determination of these substances. Good results were obtained. He recommends the tentative adoption of the method. The referee concurs.

The Committee recommends that the method be adopted tentatively with the addition of the sentence: "Examine the residues obtained qualitatively to establish their identity." The Committee also approves the recommendation of the referee that the separation of acetophenetidin, aminopyrine, caffeine, and phenobarbital be studied next year.

## ETHYL AMINO BENZOATE

No report was received. It is recommended that the subject be continued.

**SULFAPYRIDINE**

The associate referee submitted to collaborative study a method depending upon a sodium nitrite titration. Excellent results were obtained with pure sulfapyridine and fair results with mixtures of sulfapyridine and excipients. The associate referee recommends that the study of sulfapyridine be continued. The referee and the Committee concur in the recommendation.

**VEGETABLE DRUGS AND THEIR DERIVATIVES**

The referee reports that he concurs in the recommendations of the associate referees as outlined below.

**CHEMICAL METHODS FOR ERGOT ALKALOIDS**

The associate referee reports that he was unable to obtain a supply of the pure alkaloids in time to do much work on this topic this year. He recommends that the subject be continued. The Committee concurs.

**THEOPHYLLIN SODIUM SALICYLATE**

The associate referee submitted no formal report. He reports through the referee that he has revised the method reported last year, but was unable to submit samples for collaborative investigation. He and the referee recommend the continuation of this topic. The Committee approves the recommendation.

**PHYSOSTIGMINE SALICYLATE**

The associate referee submitted a method for the determination of this compound to collaborative study. He recommends the tentative adoption of the method, and also recommends that the assay of physostigmine in ointments be studied. The Committee approves both recommendations.

**ARECOLINE HYDROBROMIDE**

The associate referee devised a method, which he recommends for collaborative study. The Committee concurs.

**QUININE ETHYL CARBONATE**

No formal report was received. The associate referee reports through the referee that he was unable to do any work on this topic this year, but requests that the topic be reassigned to him. The Committee so recommends.

**THEOBROMINE AND PHENOBARBITAL**

The associate referee studied a method that gave good results with mixtures of the pure compounds, but unsatisfactory results when lactose was present. He recommends that the subject be continued. The Committee concurs.



**PLASMOCHINE**

The associate referee studied the determination of plasmochine alone and also of a mixture of plasmochine and quinine. He found that plasmochine could be determined either by the usual acid-base titration or by titration with sodium nitrite. Plasmochine and quinine could be determined when both are present by weighing the total alkaloids and then titrating the plasmochine with sodium nitrite. He recommends that the topic be continued for collaborative study. The Committee concurs.

**DRUG BIOASSAYS**

The referee states that in general, problems of biological assay do not lend themselves as well to collaborative study as do the problems of chemical assay. He proposes two subjects for study, a method for dealcoholizing tinctures of digitalis and a general study of enteric coatings.

The Committee approves the suggested projects. It does not, however, subscribe to the referee's view that bioassays are not a proper subject for collaborative study. On the contrary, it believes that the inherent inaccuracy of biological assays makes it particularly important that biological methods be submitted to collaborative study to establish whether concordant results can be obtained by different laboratories. The importance of this has already been established by the Association's own work on vitamin assay.

**MISCELLANEOUS DRUGS**

The referee's comments on the reports of the associate referees will be referred to under each individual topic. He also recommends that qualitative tests for ethyl and isopropyl alcohols and acetone be studied, as well as a new rapid method for thyroid, which is due to M. L. Yakowitz.

The Committee approves these recommendations.

**MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS**

The associate referee submitted to collaborative study methods for the detection of physostigmine, dilaudid, sulfapyridine, and sodium sulfapyridine. Results are satisfactory, and he recommends the tentative adoption of the tests. He also suggests that tests for benzedrine, metrazol, and sulfathiazol be studied next year.

The referee and the Committee concur in these recommendations.

**IODINE OINTMENT**

The associate referee submitted to collaborative study a method for the determination of iodide iodine that depends upon a titration with silver nitrate for total iodides and the use of the adsorption indicator, *p*-ethoxychrysoidin, after the free iodine has been reduced with sodium thiosulfate. Satisfactory results were obtained, and the associate referee recommends the tentative adoption of the method.

The adoption of this method necessitates a change in the reagent for

determining free iodine from potassium arsenite to sodium thiosulfate. The referee and the Committee recommend that the thiosulfate method outlined by the associate referee be substituted for the present tentative method for free iodine (p. 619, 184) and that the adsorption titration method for total iodides also be adopted as tentative. It is also recommended that this subject be closed.

#### MAGNESIUM TRISILICATE

This is a new subject. The associate referee with the help of two other analysts studied methods for loss on ignition, basicity, magnesium, silica, free alkali, soluble salts, and adsorption. He recommends continued study. The referee and the Committee concur.

#### MERCURY OINTMENT

In the report of the Committee last year it was recommended that the associate referee study the possibility of devising one uniform method for all ointments of mercury and its compounds. The associate referee reports that this would not be possible except at a loss of simplicity or accuracy. He also reports that ointment of red mercuric oxide is not now on the market, and that if it is retained in the coming edition of the National Formulary, a method of assay will be provided. He, therefore, recommends that the subject of mercury ointments be closed.

The referee and the Committee concur.

#### EMULSIONS

No report was received. It is recommended that the subject be continued.

#### COMPOUND OINTMENT OF BENZOIC ACID

The associate referee has made an intensive study of the problems involved in the analysis of this preparation. He believes that next year he will be able to submit to collaborative study a simple and accurate method for the determination of benzoic and salicylic acids, and recommends that the subject be continued.

The referee and the Committee concur.

#### ELIXIR OF THREE BROMIDES

Due to a misunderstanding, instead of working on this preparation the associate referee studied the more complicated preparation, "Sirup of the Bromides." Some collaborative work was done.

The Committee recommends that an associate referee on bromide preparations be appointed to study the elixirs of three and five bromides and sirup of the bromides. It is recommended that the method of Goldstein and Reindollar, *J. Am. Pharm. Assoc.*, 28, 85 (1939), be given consideration.

## NEW SUBJECTS

Besides the new subjects mentioned under the reports of the various referees, it is recommended that the following subjects be studied:

- (1) Applicability of the present method for barbital and phenobarbital to other barbiturates.
- (2) Aminopyrine and ephedrine.
- (3) Cinnamyl ephedrine.
- (4) Determination of mercury compounds by the ethanolamine method.
- (5) Separation of bromides, chlorides, and iodides.
- (6) Ascorbic acid.
- (7) Prostigmine.

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## REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES\*

By J. O. CLARKE (U. S. Food and Drug Administration, Chicago, Ill.),  
*Chairman*; G. G. FRARY, and W. B. WHITE

### CANNED FOODS

It is recommended—

(1) That the tentative method for the determination of total solids in tomato products (p. 520, 18) be adopted as official (first action) with elimination of the parenthetical caption.

(2) That the official method for the determination of total chlorides in tomato juice (*This Journal*, 20, 78; 21, 90; 22, 88) be dropped (final action).

(3) That the tentative rapid method for the determination of sodium chloride in tomato products (p. 521, 25) be studied collaboratively in comparison with the present official method (p. 521, 24).

(4) That studies of methods for quality factors and fill of container be continued.

(5) That the method for the determination of free and combined acid in tomato products described by the associate referee be studied collaboratively.

### COFFEE AND TEA

It is recommended—

(1) That studies be made of methods for the determination of chlorogenic acid in coffee.

### DAIRY PRODUCTS

It is recommended—

(1) That rapid methods for the direct determination of fat in butter be studied.

(2) That studies of methods for the determination of fat in malted milk

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\* These recommendations, submitted by Subcommittee C, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis*, A.O.A.C., 1940.

be continued and extended to include methods involving more complete hydrolysis of non-fat constituents.

(3) That the method for the determination of casein in malted milk described by the associate referee be adopted as tentative and subjected to further study.

(4) That studies of methods for the determination of lactic acid in dried milk and skim milk be continued.

(5) That studies of methods for the detection of neutralizers in dairy products be continued, with particular attention given to the ratio between titrable acidity and lactic acid.

(6) That the New York City laboratory phosphatase test for pasteurization of milk be adopted as tentative, as recommended by the associate referee, and that studies on phosphatase tests be continued and broadened to include cream.

(7) That the suggestion of the associate referee regarding filter paper in the present tentative phosphatase test (p. 281, 36(d) ) be adopted.

(8) That studies of methods for the detection of the use of unpasteurized cream in the manufacture of butter be postponed pending future developments.

(9) That studies be made of methods for the determination of sugars in sweetened condensed milk.

(10) That studies be continued on methods of isolating fat from cheese for the determination of fat properties and constants.

(11) That studies be made looking toward the unification of methods for the determination of total solids and ash in milk and evaporated milk.

(12) That studies on methods for the clarification of milk for the optical determination of lactose be continued and broadened to include correction for volume of the precipitate.

(13) That the method for the determination of total solids in plain ice cream suggested by the associate referee be adopted as tentative.

(14) That studies of methods of sample preparation and of analysis of frozen desserts be continued.

(15) That the tentative method for the determination of mold mycelia in butter (p. 300, 108 and 109) be adopted as official (first action).

(16) That studies on methods for the detection of decomposition in dairy products be continued.

(17) That methods for preparation of sample and sampling of cheeses be studied.

(18) That study of the stirrer method for preparation of sample of butter be continued.

#### EGGS AND EGG PRODUCTS

It is recommended—

(1) That studies of methods for unsaponifiable matter and cholesterol be continued along the lines suggested by the referee.

(2) That the tentative method for the determination of fat by acid hydrolysis (p. 310, 8) be further studied.

(3) That studies of methods for the determination of added glycerol be continued.

(4) That the rapid method for the determination of acidity of ether extract in dried eggs (*This Journal*, 15, 341) be studied by the associate referee with a view to its adoption as tentative.

(5) That the work on developing chemical methods for the detection and measurement of spoilage in eggs be continued.

#### FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the methods for the determination of ash, salt, and total nitrogen (pp. 318–19, 4–8, inclusive) be further studied collaboratively before final adoption as official.

(2) That studies of methods for the determination of total solids and ether extract be continued.

(3) That the method for the determination of volatile acids and for formic acid in canned salmon and tuna fish (*This Journal*, 21, 684, 688) be studied collaboratively.

(4) That the method for the determination of ammonia in meat (p. 375, 9) be studied collaboratively in regard to its applicability to fish and other marine products.

#### GUMS IN FOODS

It is recommended—

(1) That the tentative method for the detection of gums in soft curd cheese (p. 305, 127–130, inclusive) be subjected to collaborative study.

(2) That the tentative method for the detection of gums and dextrin in wine (p. 170, 35) be dropped.

(3) That the tentative method for the detection of gums in mayonnaise and French dressing (p. 477, 55) be studied collaboratively.

(4) That studies of methods for the detection of gums in frozen desserts be continued.

(5) That studies of methods for the detection of gums in starchy foods be undertaken.

#### MEAT AND MEAT PRODUCTS

It is recommended—

(1) That studies of methods for the detection and determination of dried skim milk and soy bean flour be continued.

(2) That the subject of metals in gelatin be transferred to the Referee on Metals in Foods.

#### METALS IN FOODS

It is recommended—

(1) That studies be continued on methods of preliminary treatment of those products in which the arsenic is tenaciously held.

(2) That the iodine titration, gold or silver sol, and the molybdenum blue colorimetric methods for the determination of arsenic be further studied as possible substitutes for the Gutzeit method.

(3) That in the studies of methods for the determination of antimony and of arsenic, special attention be given to the separation of micro quantities of these elements occurring simultaneously in organic or biological material.

(4) That studies on micro methods for the determination of copper be continued along the lines suggested by the referee.

(5) That studies be continued on methods for the determination of fluorine.

(6) That the editorial change in the official rapid method for the determination of lead (pp. 407-9, 30, 31, and 32) suggested by the associate referee be made in order to adapt it to the liberalized lead tolerance.

(7) That studies on the determination of lead be continued and extended to include gelatin.

(8) That studies on the determination of mercury be continued.

(9) That studies be continued on methods for the determination of selenium.

(10) That the tentative method for zinc (p. 415, 46 and 47) be further studied, and that the various workers on zinc determination be urged to cooperate.

(11) That studies be continued on micro and macro methods for the determination of hydrocyanic acid.

#### MICROBIOLOGICAL METHODS

It is recommended—

(1) That the tentative methods for the examination of frozen egg products be revised as suggested by the associate referee and further studied.

(2) That the methods for canned tomatoes and other acid vegetable and fruit products suggested by the associate referee be adopted as tentative and further studied collaboratively.

(3) That studies be continued on canned vegetables, canned fishery products, canned meats, frozen eggs, and sugar.

(4) That studies be initiated on bacteriological methods for frozen fruits and frozen vegetables and for nuts and nut products.

#### OILS, FATS, AND WAXES

It is recommended—

(1) That study of the tentative method for determining the thiocyanogen number of fats and oils (p. 431, 22) be discontinued.

(2) That studies be made on the application of the official method for refractometric determination of oil in flaxseed (pp. 448-52, 63-65, inclusive) to other oil seeds.

(3) That studies on the Polenske method be continued.

(4) That methods for the determination of olive oil in admixture with other oils be developed.

(5) That the modifications of the titer test approved by the American Chemical Society and the American Oil Chemists' Society be studied.

(6) That methods for the determination of unsaponifiable matter be studied with a view to adopting a method of wide applicability.

#### SPICES AND CONDIMENTS

It is recommended—

(1) That studies on the determination of total solids in vinegar be continued with special reference to vinegars high in solids.

(2) That in view of the results obtained by the associate referee as well as by earlier workers, the procedure suggested by the associate referee for the determination of soluble and insoluble phosphoric acid in vinegar be adopted as tentative and that the present official methods (p. 478, 63 and 64) be dropped (first action).

(3) That the method for the determination of total phosphoric acid in vinegar suggested by the associate referee be adopted as tentative.

(4) That the method for the detection of caramel in vinegar described by the associate referee be studied collaboratively.

(5) That the "oxygen value" method for differentiating between distilled vinegar and commercial acetic acid described by the associate referee be studied collaboratively, and that studies be initiated on other methods for such differentiation.

(6) That the tentative method for preparation of sample of mayonnaise and salad dressing (p. 475, 44) be modified as suggested by the associate referee and subjected to further study.

(7) That the tentative method for the determination of total fat in mayonnaise and salad dressing (p. 476, 52) be modified to provide for a 1 gram sample and subjected to further study.

(8) That the tentative methods for sugars (pp. 475-6, 46-48) and for identification of the oil in mayonnaise and salad dressing (p. 477, 54) be adopted as official (first action).

(9) That studies be initiated on methods for the determination of starch in salad dressings.

(10) That the tentative method for the determination of volatile oil in spices (pp. 469-70, 16) be further studied.

(11) That the method for the determination of starch in mustard flour and prepared mustard described by the associate referee be studied collaboratively.

(12) That the official method for the determination of volatile oil in mustard seed (p. 472, 25) be studied in regard to its application to different types and to other mustard products.

(13) That the method for the direct determination of moisture in

spices suggested by the referee be adopted as tentative and subjected to collaborative study.

(14) That studies on direct titration methods for the determination of moisture in spices be continued.

(15) That the method for the determination of ash in spices (p. 468, 3) be dropped (first action).

(16) That the method for the determination of ash in spices described by the referee be adopted as tentative and subjected to collaborative study.

(17) That methods for the determination of ash in prepared mustard be studied.

(18) That the method for determining the iodine number of paprika oil (p. 472, 27) be made official (final action).

(19) That studies be made of methods for the determination of salt in prepared mustard.

#### FOOD PRESERVATIVES AND SWEETENERS

It is recommended—

(1) That studies be continued on methods for the determination of benzoate of soda in foods.

(2) That the qualitative test presented by the referee for the determination of saccharin in nonalcoholic beverages, semi-solid preparations, and baked goods be adopted as tentative.

(3) That methods for the determination of the esters of benzoic acids be studied.

#### COLORING MATTER IN FOODS

It is recommended—

(1) That collaborative work be continued on the method for the determination of FD&C Red No. 4 (Ponceau SX) in the presence of FD&C Red No. 1 (Ponceau 3R).

(2) That investigational work be continued on the quantitative separation and estimation of FD&C Yellow No. 5 (Tartrazine) and FD&C Yellow No. 6 (Sunset Yellow FCF).

(3) That investigational work be undertaken to separate and determine quantitatively mixtures of FD&C Green No. 2 (Light Green SF Yellowish), FD&C Green No. 3 (Fast Green FCF), and FD&C Blue No. 1 (Brilliant blue FCF).

(4) That collaborative work on analytical methods for the coal-tar colors certifiable for use in foods be conducted.

#### MICROCHEMICAL METHODS

It is recommended—

(1) That the methods for the determination of nitrogen presented by the referee be adopted as tentative.

(2) That studies of microchemical methods be continued.

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## REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES\*

By W. C. JONES (Department of Agriculture, Richmond, Va.),  
Chairman; J. W. SALE, and J. A. LECLERC

### SUGAR AND SUGAR PRODUCTS

It is recommended—

(1) That the collaborative work on the determination of unfermented reducing substances in molasses be repeated by next year's referee, that the Quisumbing-Thomas method be used for copper reduction, and that the copper in the precipitate be determined volumetrically.

(2) That the procedure for the determination of diacetyl given in this year's report be subjected to collaborative study.

(3) That study of drying, densimetric, and refractometric methods be continued.

(4) That studies on the determination of moisture in honey be continued, and that studies on honey-dew honey be inaugurated.

(5) That work be done on sucrose and ash in molasses.

(6) That the work on refractive indices of invert sugar solutions and the change in refractive indices with change of temperature in such products as invert sugar solutions, table sirups, etc., be continued.

### WATERS, BRINE, AND SALT

It is recommended—

(1) That the method for the determination of fluorine in water presented last year, with the changes suggested and discussed this year, be adopted as official (first action).

(2) That study on the determination of moisture in effervescent salts be discontinued.

(3) That studies on the determination of boron in water be continued.

### CACAO PRODUCTS

It is recommended—

(1) That work on the determination of lecithin in cacao products be continued.

(2) That the proposed method for the determination of milk protein in milk chocolate be given further collaborative study with the use of mercury alone as catalyst.

(3) That further collaborative work be done on the method for the determination of pectic acid in cacao products.

(4) That the procedure given in the report of the Referee on Chocolate in Sweet and Milk Chocolate be adopted as a tentative method for the determination of the chocolate constituent of sweet chocolate (with or

\* These recommendations, submitted by Subcommittee D, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis*, A.O.A.C., 1940.

without small quantities of added dairy ingredients), milk chocolate, mixed milk chocolate, skim milk chocolate, buttermilk chocolate, cocoa and fat (other than cacao fat), coatings, and chocolate and fat (other than cacao fat) coatings.

#### ALCOHOLIC BEVERAGES

##### MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS

It is recommended—

(1) That the available collaborative results of the study of the electro-metric method for pH be submitted with a view to its adoption as official (first action) next year.

(2) That the following subjects be further studied: Total acidity by potentiometric titration, chloride, dextrans, sulfur dioxide, and metals such as tin and copper.

(3) That the study of methods for proteolytic activity of malt be continued.

(4) That further studies be made of the method for the determination of iron in beer outlined in the associate referee's report.

(5) That the study of methods for the analysis of malt adjuncts be continued.

(6) That the subject of hops be studied.

(7) That special study be made of the diastatic activity of malt.

(8) That the tentative manometric method for the determination of carbon dioxide in beer be further studied collaboratively with the object of making it official (first action).

(9) That study on malt extract in malt be continued.

#### WINES

It is recommended—

(1) That further studies be made on the determination of sulfur dioxide in wines.

(2) That the effect of lactic acid on the volatile acidity of wines be reinvestigated in regard to the various types of apparatus now in use.

#### DISTILLED LIQUORS

It is recommended—

(1) That the colorimetric method for the determination of tannins presented by the Associate Referee on Whiskey and Rum be adopted as tentative.

(2) That methods for the determination of methanol and denaturants in distilled spirits be studied.

(3) That study of methods for the analysis of whiskey and rum be continued.

(4) That the method for the determination of benzaldehyde (p. 184, 63, 64) be adopted as official (final action).

(5) That the method for the determination of volatile esters in cordials (p. 183, 60) be adopted as official (final action).

(6) That the method for the determination of gamma-undecalactone (qualitative), (p. 183, 61) be adopted as official (final action).

(7) That the method for the determination of total solids from refractive index of dealcoholized sample (p. 181, 44(c)), be adopted as official (final action).

(8) That study on cordials and liqueurs be continued.

#### FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That further study be made of the determination of sodium and chlorine.

(2) That further study of polariscopic methods be made.

(3) That electrometric titration methods for total acidity be studied.

(4) That the colorimetric method for the determination of phosphoric acid (p. 347, 39, 40, 41) be adopted as official (first action).

(5) That a comparative study be made of the gravimetric method (p. 22, 9), volumetric method (p. 22, 12), and colorimetric method (p. 347, 39-41) for the determination of phosphoric acid.

(6) That the cobaltinitrite procedure for the determination of  $K_2O$  be further studied.

(7) That collaborative study be made of the method proposed by the referee for the determination of citric acid.

(8) That collaborative study be made of the method for the determination of lactic acid given in *This Journal*, 20, 605 (1937).

(9) That further study be made of methods for the determination of other fruit acids.

(10) That rapid control methods for potassium be studied.

(11) That methods for the examination of cold pack fruit be studied.

#### FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That the method for the determination of  $\beta$ -ionone (p. 332, 68) be adopted as official (final action).

(2) That the method for the determination of  $\beta$ -ionone in raspberry flavors (pp. 332-3, 69, 70) be adopted as official (final action).

(3) That the directions submitted by the associate referee for a more permanent standard vanillin solution be added under reagents (p. 321, 6).

(4) That the associate referee continue the studies of organic solvents in flavors.

(5) That work on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanillas be continued, with special reference given to automatic extraction of vanillin and coumarin and the spectrophotometric method for coumarin.

(6) That the method for the determination of  $\beta$ -ionone when 1–10 mg. is present (*This Journal*, 22, 691), as applied to beverages, be submitted to collaborative study.

(7) That collaborative study be made of the application to lemon oils and extracts of the Ripper method for the determination of aldehydes in spirits.

(8) That collaborative study be made of the application to emulsion flavors of the official steam distillation method for the determination of lemon, orange, and lime oils in vegetable and mineral oils.

(9) That studies on maple concentrates and imitations be continued.

#### CEREAL FOODS

It is recommended—

(1) That the colorimetric method for the determination of H-ion concentration of cereal products, with sulfonphthalein indicators (p. 213, 14) be made official (final action) and that further work be done to establish a satisfactory procedure for the determination of hydrogen ion of cereal products by an electrometric method.

(2) That further study be given to methods for the determination of starch in raw and cooked cereals.

(3) That further study be given to the determination of fat acidity in grain, flour, corn meal, and whole wheat flour, and to the correlation of fat acidity with unsoundness of corn meal and whole wheat flour.

(4) That further study be given to the determination of sugar in flour by the method submitted by the associate referee and to its application to the determination of sugar in bread and other cereal products.

(5) That the study of the baking test for soft wheat flour be continued.

(6) That study of the method for the determination of chlorine in the fat of flour (p. 222, 40) be continued.

(7) That the tentative method for the determination of benzoyl peroxide bleach in flour (p. 223, 44) be further studied.

(8) That methods for the determination of carotenoid pigments in flour be further studied.

(9) That methods for the determination of carbon dioxide in self-rising flour (p. 212, 9) be further studied.

(10) That the lactose procedure for the estimation of milk solids in bread, with any necessary modifications (*Cereal Chem.*, 13, 541) be further studied collaboratively.

(11) That study of the estimation of butterfat in bread be continued.

(12) That the study of proteolytic activity of flour recommended by the associate referee be continued.

(13) That the study of color measurements of flour and bread be discontinued until these methods are more definitely established.

(14) That the study of methods for the determination of cellulose in whole-wheat-flour products be continued.

(15) That further study be made of the official methods for the determination of moisture, ash, protein, crude fiber, and fat in flour in regard to their applicability to rye, oats, corn, and buckwheat products.

(16) That the methods for the determination of moisture, ash, protein, crude fiber, and fat in flour (p. 211, 2; 212, 5; 213, 12; 213, 10 and 11) be adopted as tentative for the analysis of rice and barley products and that study be continued.

(17) That the study be continued on methods for the determination of moisture, ash, protein, fat, and crude fiber in bakery products, including those containing fruits.

(18) That further study be given to the determination of moisture in self-rising flour and pancake, waffle, and doughnut flour.

(19) That studies be continued on methods for the identification of the raw materials used in the manufacture of macaroni.

(20) That the method described by the associate referee for the determination of unsaponifiable matter in noodles and the farinaceous ingredients of noodles be adopted as official (first action).

(21) That the method developed by the associate referee for the determination of sterols in noodles and the farinaceous ingredients of noodles be adopted as official (first action).

(22) That the methods referred to in Recommendations 20 and 21 be applied to other farinaceous egg-containing products and further studied collaboratively.

(23) That the study of cold water extract in flour be discontinued.

(24) That the study of phosphated flour be continued.

(25) That the study of soya flour in foods be continued, and that the associate referee study the applicability to soya flour of methods applicable to wheat flour, especially those for the determination of water, ash, protein, fat, and fiber.

(26) That an associate referee be appointed to study rye flour in rye bread and in flour mixtures.

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## CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FIFTY-SIXTH ANNUAL MEETING, OCTOBER 28, 29, AND 30, 1940\*

### I. SOILS

No additions, deletions, or other changes.

### II. FERTILIZERS

(1) The beaker method for the determination of water-insoluble nitrogen (35, p. 29) was adopted as official (final action).

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\* Compiled by Marian E. Lapp, *Associate Editor*. Unless otherwise given, all references in this report are to *Methods of Analysis*, A.O.A.C., 1940, and the methods are edited to conform to the style used in that publication.

(2) The following directions were included in the parentheses of the last line of 42(a) of the Lindo-Gladding method for the determination of potash (p. 31): "If not, dissolve the  $K_2PtCl_4$  in hot  $H_2O$ , reweigh, and make correction for water-insoluble residue."

(3) The following grindings and sieves were adopted for the grinding of samples:

For fertilizer materials and moist fertilizer mixtures, to pass a sieve with 1 mm. circular openings, or 20-mesh Tyler standard sieve; dry mixtures that show a tendency to segregate may be ground to pass a 35-mesh Tyler standard sieve.

(4) The following method for the determination of acid-soluble calcium in mixed fertilizers was adopted as tentative:

#### ACID-SOLUBLE CALCIUM IN MIXED FERTILIZERS

Place the Ca oxalate and filter paper from acid-soluble magnesium (p. 36, 52) in beaker in which precipitation was made and add a mixture of 125 ml of  $H_2O$  plus 5 ml of  $H_2SO_4$ . Heat to  $70^\circ$  or above and titrate with 0.1 N  $KMnO_4$  until first slight pink color appears. Correct for blank and calculate to Ca.

(5) The tentative method for the determination of acid-soluble calcium (p. 34, 47) was changed to read as follows:

Weigh 2.5 g of sample into 250 ml volumetric flask, add 30 ml of  $HNO_3$  and 10 ml of  $HCl$ , and boil 30 min. Cool, make to volume, and mix. Filter if necessary. Transfer to a beaker a 25 ml aliquot of dissolved sample and dilute to 100 ml. Add 2 drops of bromophenol blue indicator. Add  $NH_4OH$  (1 + 4) to the point where the indicator changes from yellow to green (not blue). If over-run, bring back with  $HCl$  (1 + 4). (This gives pH of 3.5–4.0.) Dilute to 150 ml. Bring to boiling and add slowly with constant stirring 30 ml of saturated hot  $NH_4$  oxalate soln. If the color changes from green to blue or yellow again, adjust to green with the  $HCl$  (1 + 4). Digest on steam bath 1 hour, or let stand overnight, and cool to room temp. Filter supernatant liquid through quantitative filter paper on Gooch crucible or on fritted glass filter, and wash precipitate thoroughly with  $NH_4OH$  (1 + 50). Place filter paper or crucible with precipitate in original beaker and add mixture of 125 ml of  $H_2O$  plus 5 ml of  $H_2SO_4$ . Heat to  $70^\circ$  or above and titrate with 0.1 N  $KMnO_4$  until first slight pink color is obtained. Correct for blank and calculate to Ca.

(6) The tentative volumetric method for the determination of copper (p. 34, 50, 51) was revised by the associate referee. The details of the method, which remains as tentative, follow:

#### COPPER

##### Volumetric Method

##### REAGENTS

(a) *Standard sodium thiosulfate soln.*—Dissolve 7.82 g of  $Na_2S_2O_3 \cdot 5H_2O$  in  $H_2O$  and dilute to 1 liter.

(b) *Standard copper nitrate soln.*—Place 2.000 g of pure Cu (electrolytic) in 1 liter volumetric flask, add 100 ml of  $HNO_3$ , heat until the Cu is dissolved, and dilute with  $H_2O$  to volume at room temp.

(c) *Potassium iodide.*—Dissolve 50 g of KI in enough  $H_2O$  to make 100 ml of soln.

(d) *Starch soln.*—Mix ca 1 g of soluble starch with enough cold  $\text{H}_2\text{O}$  to make a thin paste, add 100 ml of boiling  $\text{H}_2\text{O}$ , and boil while stirring for ca 1 min.

#### DETERMINATION

Weigh 2 g of sample if less than 5% Cu. If more than 5% Cu, weigh sufficient quantity to furnish a little less than 0.1000 g of Cu. Place sample in 300 ml Erlenmeyer flask, and add 5–10 ml of  $\text{HNO}_3$  and 7.0 ml of  $\text{H}_2\text{SO}_4$ . Digest on hot plate to dense white fumes. If soln becomes dark due to organic matter, cool somewhat, add a little more  $\text{HNO}_3$ , and digest again to dense white fumes, repeating the operation if necessary until the organic matter appears to be destroyed. Cool, and add 25–30 ml of  $\text{H}_2\text{O}$ . Boil 1 min., remove from hot plate, and stir occasionally for ca 15 min. Filter into 250 ml Erlenmeyer flask and wash filter and residue 6 times with small portions of hot  $\text{H}_2\text{O}$ . Cool to room temp. and dilute to 100 ml.

Pass  $\text{H}_2\text{S}$  through the soln in an Erlenmeyer flask for 10–15 min. Prepare a wash soln of 10 ml of  $\text{H}_2\text{SO}_4$ , plus enough  $\text{H}_2\text{O}$  to make 1 liter, and saturate with  $\text{H}_2\text{S}$ . Filter sample soln through a paper of fine texture and wash paper and precipitate 7 times with small portions of the wash soln, keeping filter funnel covered with watch-glass as much of time as possible. Reserve filtrate for Zn determination.

Place paper and precipitate in glazed porcelain crucible and ignite at dull red heat until C is completely destroyed. Blow the  $\text{H}_2\text{S}$  gas out of precipitation flask and wash the  $\text{CuS}$  from the  $\text{H}_2\text{S}$  delivery tube into flask with bromine  $\text{H}_2\text{O}$ . Add 5 ml of  $\text{HNO}_3$  to the  $\text{CuO}$  in cold crucible and warm until the  $\text{CuO}$  is dissolved. (This may require 10 min., at end of which time insoluble specks may be disregarded.) Wash soln into precipitation flask with  $\text{H}_2\text{O}$  and dilute to 35 ml. For standardizing the  $\text{Na}_2\text{S}_2\text{O}_3$  soln add to another 250 ml Erlenmeyer flask an aliquot of the standard  $\text{Cu}(\text{NO}_3)_2$  soln and more  $\text{HNO}_3$ , so that an equivalent of 5 ml of  $\text{HNO}_3$  shall be present, and dilute to 35 ml. Hereafter treat all solns alike. Add an excess of bromine  $\text{H}_2\text{O}$  and a few glass beads. Boil until excess of bromine is entirely expelled and volume is less than 30 ml. Cool a little and add  $\text{NH}_4\text{OH}$  cautiously until mixture is distinctly alkaline. Boil until the odor of  $\text{NH}_3$  is very faint. Add 5 ml of glacial acetic acid and boil a min. more. Cool to room temp. and dilute to 25–30 ml. Add 2 ml of the  $\text{KI}$  soln and titrate with the  $\text{Na}_2\text{S}_2\text{O}_3$  soln to a light yellow color. Add ca 1 ml of the cold starch soln and continue titration to disappearance of starch-iodine color. Calculate Cu equivalent of thiosulfate soln from titration of the soln containing the known amount of Cu and from this factor calculate amount of Cu in the sample soln.

(7) The following gravimetric method for the determination of zinc was adopted as tentative:

#### ZINC

##### *Gravimetric Method*

(Maximum 0.20 per cent zinc)

Evaporate combined filtrate and washings from the  $\text{CuS}$  precipitation (preceding method) in 250 ml Erlenmeyer flask to ca 80 ml. Cool, and add 2 drops of 0.4% bromophenol blue soln and then  $\text{NH}_4\text{OH}$  from dropper to first distinct color change of indicator. Cool to room temp. and add 10 ml of 20% citric acid soln. Fit flask with a two-holed rubber stopper and glass tubes, one of which almost touches bottom of flask and other just extends through stopper. Pass rapid stream of  $\text{H}_2\text{S}$  through soln for 45 min. Prepare soln containing 0.5 g of citric acid per liter and saturate with  $\text{H}_2\text{S}$ . Filter sample soln through ashless paper of fine texture. Use a rubber policeman to loosen precipitate sticking to flask and delivery tube and wash onto filter with a jet of the prepared wash soln. Wash paper and precipitate 7 more times with small quantities of the wash soln, keeping funnel covered with watch-glass as much of time as possible. Place paper and precipitate in crucible that has been ig-

nited and weighed with cover. Ignite in the uncovered crucible at a low temp., preferably in muffle, until paper is oxidized, then at 900–950° for 1 hour. Place cover on crucible while hot, cool in desiccator, and weigh as ZnO. Calculate to Zn.

(8) The method for the determination of magnesium in water-soluble compounds (p. 36, 55) was adopted as official (final action).

(9) Method II for the determination of acid-soluble magnesium (p. 36, 53, and *This Journal*, 22, 270; 23, 247), was modified as follows and adopted as official (first action).

In the 5th line from end of method the sentence "Stir vigorously until precipitate is complete," was changed to read: "Stir vigorously until precipitate forms." and in the 3rd line from end of method after the clause "or allow to stand overnight," the following sentence was inserted: "(If only very small quantities of Mg are present, and no precipitate forms during stirring or after adding 15 ml of  $\text{NH}_4\text{OH}$ , allow to stand overnight.)"

(10) Method III, volumetric modification of the method for the determination of acid-soluble magnesium (p. 36, 54) was adopted as official (first action).

(11) The colorimetric method for the determination of acid-soluble manganese (*This Journal*, 22, 279; 23, 250) was adopted as official (first action).

(12) The following bismuthate method for the determination of acid-soluble manganese was adopted as tentative:

#### ACID-SOLUBLE MANGANESE

##### *Bismuthate Method*

##### REAGENTS

(a) *Sodium bismuthate powder*.—80%  $\text{NaBiO}_3$ , containing not more than 0.0005% Mn, and not more than 0.002% of Cl.

(b) *Potassium permanganate*.—0.0910 N/2.876 g of  $\text{KMnO}_4$  in 1 liter of soln. 1 ml contains 1 mg. of Mn. Standardize with Na oxalate.

(c) *Ferrous sulfate*.—0.091 N/25.3 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 25 ml of  $\text{H}_2\text{SO}_4$ , and 25 ml of 85%  $\text{H}_3\text{PO}_4$  in 1 liter of soln. 1 ml = 1 mg of Mn. Standardize with  $\text{KMnO}_4$  near the time of actual use. Place measured portion approximately equivalent to maximum quantity of Mn to be determined in Erlenmeyer flask containing 200 ml of cold  $\text{H}_2\text{SO}_4$  (3+97), and titrate with the  $\text{KMnO}_4$  soln.

##### DETERMINATION

To 1 g sample in Erlenmeyer flask (preferably 300 ml), add 5–10 ml of  $\text{HNO}_3$ , and 7 ml of  $\text{H}_2\text{SO}_4$ . Evaporate on a hot plate to white fumes. Add a few drops of  $\text{HNO}_3$ , again evaporate to white fumes, and repeat until organic matter is destroyed. Cool. Add 100 ml of  $\text{H}_2\text{O}$ , 10 ml of  $\text{HNO}_3$ , and just enough  $\text{NaBiO}_3$  to give soln a strong permanganate color, or in case of small quantity of Mn, a slight excess of  $\text{NaBiO}_3$ . Boil gently 2–3 min. If the permanganate color or  $\text{MnO}_2$  disappears, cool somewhat, and repeat bismuthate treatment. A permanent permanganate color or the persistence of  $\text{MnO}_2$  indicates a sufficient excess of bismuthate. Add a saturated soln of  $\text{NaHSO}_3$  dropwise while stirring until Mn compounds are reduced and soln clears. Avoid a large excess. Boil gently 2–3 min. Cool to room temp. and make to a volume of ca 100 ml. If soln contains less than 40 mg of Mn, proceed with determination. If more than 40 mg of Mn is present, transfer to 200 ml volumetric



flask, add 5 ml of  $H_2SO_4$  and 10 ml of  $HNO_3$ , cool, dilute to volume, and mix. Pipet an aliquot containing not more than 40 mg of Mn into Erlenmeyer flask and dilute to 100 ml with  $H_2O$  soln containing 5 ml of  $H_2SO_4$  and 10 ml of  $HNO_3$  in 100 ml.

Before continuing, prepare suction filters of asbestos washed with the  $H_2SO_4$  and then with  $H_2O$ . (Glass filter tubes with perforated porcelain disks to support asbestos and connected with suction flask are satisfactory. Mn soln must not come in contact with rubber.) From this point complete determination without interruption. To the Mn soln at 20–30° add at least 0.25 g of  $NaBiO_3$  for each 10 mg of Mn. (It may be measured by weight or by volume of known approximate weight relationship.) Swirl contents of flask for 1 min., add 100 ml of  $H_2O$ , and mix. Filter with suction through prepared filter and wash with cold  $H_2SO_4$  (3+97) until washings show no pink tint. Disconnect suction flask and from buret add the  $FeSO_4$  soln until permanganate color disappears; then add at least 10% in excess with 1 ml as minimum excess. Titrate excess  $FeSO_4$  with the  $KMnO_4$  soln to faint pink. From the  $KMnO_4$  equivalent to the ml of  $FeSO_4$  soln used, subtract the  $KMnO_4$  used in back titration. From difference calculate percentage of Mn in sample.

### **III. SEWAGE\***

#### **IV. AGRICULTURAL LIMING MATERIALS**

No additions, deletions, or other changes.

#### **V. AGRICULTURAL DUST\***

#### **VI. INSECTICIDES AND FUNGICIDES**

(1) The following changes were made in the lead chlorofluoride method for the determination of total fluorine (p. 49, 19):

(a) Line 2, after the word "silica" insert the clause, "Cover with a heavy layer (2–3 g) of the alkali carbonates."

(b) Line 14, delete "once or twice" and insert "three times."

(c) Line 26, after parentheses, insert words, "and for 1 hour in ice bath, or keep overnight in refrigerator."

(2) The mercury reduction method for the determination of Pyrethrin I in pyrethrum powder (p. 66, 112–113) was adopted as official (final action).

(3) The crystallization method for the determination of rotenone in derris and cube powder (p. 64, 110) was adopted as official (final action).

(4) The tentative method for the determination of total ether extract in derris and cube powder (p. 66, 111) was adopted as official (final action).

#### **VII. CAUSTIC POISONS**

No additions, deletions, or other changes.

#### **VIII. NAVAL STORES**

No additions, deletions, or other changes.

#### **IX. PAINTS, VARNISHES, AND CONSTITUENT MATERIALS**

No additions, deletions, or other changes.

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\* Subjects for future study.

## X. LEATHERS

No additions, deletions, or other changes.

## XI. TANNING MATERIALS

No additions, deletions, or other changes.

## XII. PLANTS

(1) The following method for the determination of zinc in plants was adopted as tentative:

### ZINC

#### REAGENTS

(All  $H_2O$  must be redistilled from Pyrex glass. All glassware must be rinsed with strong acid and then thoroly rinsed with Zn-free  $H_2O$ .)

(a) *Carbon tetrachloride*.—Use C. P. grade without purification. If Tech. grade is used, dry with anhydrous  $CaCl_2$  and redistil in presence of small quantity of  $CaO$ . (Used  $CCl_4$  may be reclaimed by distillation in presence of  $NaOH$  (1 + 100) containing small quantities of  $Na_2SiO_3$ , followed by drying with anhydrous  $CaCl_2$  and fractional distillation in presence of small quantities of  $CaO$ .)

(b) *Standard zinc solns*.—(1) *Stock soln (1000 gamma of Zn per ml)*.—Place 0.25 g of pure Zn in 250 ml volumetric flask. Add ca 50 ml of  $H_2O$  and 1 ml of  $H_2SO_4$ , then heat on steam bath until all Zn is dissolved. Dilute to 250 ml and store in Pyrex vessel. (2) *Standard soln (10 gamma of Zn per ml)*.—Dilute 10 ml of stock soln to 1 liter. Store in Pyrex vessel.

(c) *Ammonium hydroxide soln*.—Normal. With an all-Pyrex glass apparatus distil into  $H_2O$  one-half of a volume of  $NH_4OH$  and dilute to proper concentration. Store in glass-stoppered Pyrex vessel.

(d) *Hydrochloric acid*.—Normal. Displace  $HCl$  gas from a volume of concentrated  $HCl$  in a glass flask by slow addition of equal volume of concentrated  $H_2SO_4$  by means of a dropping funnel that extends below surface of the concentrated  $HCl$ . Absorb displaced  $HCl$  gas by conducting it thru a delivery tube to surface of a volume of  $H_2O$ . (No heat is necessary.) Dilute to proper concentration. 150 ml of each of the acids will yield 1 liter of purified  $HCl$  soln of concentration greater than normal.

(e) *Diphenylthiocarbazone (dithizone)*.—Dissolve 0.20 g of dithizone in 500 ml of  $CCl_4$ , and filter soln to remove insoluble matter. Place soln in glass-stoppered bottle or large separatory funnel, add 2 liters of 0.02  $N$   $NH_3$  (40 ml of normal  $NH_3$ , diluted to 2 liters), then shake to extract the dithizone into the aqueous phase. Separate phases, discard  $CCl_4$  phase, and extract ammoniacal soln of dithizone with 100 ml portions of  $CCl_4$  until the  $CCl_4$  extract is a pure green color. Discard the  $CCl_4$  phase after each extraction. Add 500 ml of  $CCl_4$  and 45 ml of normal  $HCl$  and shake to extract the dithizone into the  $CCl_4$ . Separate phases and discard aqueous phase. Dilute the  $CCl_4$  soln of dithizone to 2 liters with  $CCl_4$ . Store in a brown bottle in dark, cool place.

(f) *Ammonium citrate soln*.—0.5  $M$ . Dissolve 226 g of  $NH_4$  citrate,  $(NH_4)_3H_2C-H_5O_7$ , in 2 liters of  $H_2O$ . Add concentrated  $NH_3$  until soln has  $pH$  of 8.5–8.7 (80–85 ml). Add excess of the dithizone (orange-yellow coloration in aqueous phase after shaking and separation of phases), and extract with 100 ml portions of  $CCl_4$  until extract is a full green color. Add more dithizone if necessary. Separate aqueous phase from the  $CCl_4$ , and store in Pyrex vessel.

(g) *Carbamate reagent*.—Dissolve 0.25 g of  $Na$  diethyldithiocarbamate in  $H_2O$  and dilute to 100 ml with  $H_2O$ . Prepare a fresh soln just before use.

(h) *Hydrochloric acid*.—0.02 *N*. Dilute 100 ml of normal HCl to 5 liters.

To reduce measuring out reagents and minimize errors due to variations in composition, prepare 3 solns in quite large quantities from the reagents and store in Pyrex vessels, taking care to avoid loss of  $\text{NH}_3$  from Solns A and B. Discard solns after they have been stored 6–8 weeks because the Zn increases slowly with storage. Determine a standard curve for each new set of reagents. The quantity of reagents designated and 2 liters of dithizone reagent are sufficient for 100 determinations.

(1) *Solution A*.—Dilute 1 liter of 0.5 *M*  $\text{NH}_4$  citrate and 140 ml of normal  $\text{NH}_4\text{OH}$  to 4 liters.

(2) *Solution B*.—Dilute 1 liter of 0.5 *M*  $\text{NH}_4$  citrate and 300 ml of normal  $\text{NH}_4\text{OH}$  to 4.5 liters. Just before using add 1 volume of freshly prepared carbamate reagent to 9 volumes of the  $\text{NH}_3\text{-NH}_4$  citrate soln to obtain volume of Soln B immediately required.

If reagents purified from Zn have been prepared, they can be used to test chemicals for Zn. It was found that certain lots of  $\text{NH}_4\text{OH}$  and HCl are sufficiently free of Zn to be used in this method without purification.

#### ASHING

Ash 5 g sample of the finely ground, air-dry plant material in a Pt dish in an electric muffle at 500–550°. Wet ash with a little  $\text{H}_2\text{O}$ , then add 10 ml of the normal HCl (more if necessary) and heat on steam bath until all substances soluble in HCl are brought into soln. Add 5–10 ml of hot  $\text{H}_2\text{O}$ . Filter off insoluble matter on a 7 cm filter paper (Whatman No. 42 or equivalent) that has been washed with two 5 ml portions of hot normal HCl, then washed with hot  $\text{H}_2\text{O}$  until free of HCl, and collect filtrate in 100 ml volumetric flask. Wash filter with hot  $\text{H}_2\text{O}$  until washings are no longer acid to methyl red. Add drop of methyl red indicator to filtrate in 100 ml flask and normal  $\text{NH}_4\text{OH}$  until neutral to methyl red, then add 4 ml of normal HCl. Allow contents of the flask to cool, then make to volume with  $\text{H}_2\text{O}$ .

#### FIRST EXTRACTION

(Separation of dithizone complex-forming metals from ash soln.)

Pipet aliquot of the ash soln containing not more than 30 gamma of Zn into a 125 ml Squibb separatory funnel. Add 1 ml of 0.2 *N* HCl for each 5 ml of ash soln less than 10 ml taken or 1 ml of 0.2 *N*  $\text{NH}_4\text{OH}$  for each 5 ml over 10 ml taken. (A 10 ml aliquot has usually been found satisfactory in the analysis of plant materials.) Add 40 ml of Soln A and 10 ml of the dithizone reagent. Shake vigorously for 30 seconds to extract from the aqueous phase the Zn and other dithizone complex-forming metals that may be present, then allow layers to separate. At this point an excess of dithizone (indicated by orange or yellow-orange coloration of aqueous phase) must be present. If excess dithizone is not present, add more of the reagent until after shaking an excess is indicated. Shake down the drop of  $\text{CCl}_4$  extract from surface, and draw off the  $\text{CCl}_4$  extract into second separatory funnel as completely as possible without allowing any of aqueous layer to enter stopcock bore. Rinse down the  $\text{CCl}_4$  extract from surface of the aqueous layer with 1–2 ml portion of clear  $\text{CCl}_4$ , then run off this  $\text{CCl}_4$  into second funnel without permitting aqueous phase to enter stopcock bore. Repeat this rinsing process as many times as necessary to completely flush extract into second funnel. Add 5 ml of clear  $\text{CCl}_4$  to first funnel, shake 30 seconds, then allow layers to separate. (The  $\text{CCl}_4$  layer at this point will have clear green color if the metals that form dithizone complexes have been completely extracted from aqueous phase by previous extraction.) Run off  $\text{CCl}_4$  layer into second funnel, then flush down extract from surface and out of funnel as directed previously. If last extract does not possess a distinct clear green color, repeat the extraction

with 5 ml portion of clear  $\text{CCl}_4$  and the flushing out process until complete extraction of the dithizone complex-forming metals is assured, then discard aqueous phase.

#### SECOND EXTRACTION

(Separation of copper by extraction of Zn into 0.02 *N* HCl.)

Pipet 50 ml of 0.02 *N* HCl into the separatory funnel containing the  $\text{CCl}_4$  soln of metal dithizonates. Shake vigorously for 1.5 min., then allow layers to separate. Shake down the drop from surface of aqueous phase, and as completely as possible run off the  $\text{CCl}_4$  phase that contains all the copper as dithizonate, without allowing any of aqueous phase, which contains all the Zn, to enter the stopcock bore. Rinse down  $\text{CCl}_4$  extract from surface of aqueous phase and rinse out stopcock bore with 1-2 ml portions of clear  $\text{CCl}_4$  (same as in the first extraction) until all traces of green dithizons have been washed out of funnel. Shake down the drop of  $\text{CCl}_4$  from surface of aqueous phase, and run off  $\text{CCl}_4$  as completely as possible without allowing any aqueous phase to enter stopcock bore. Remove stopper from funnel and lay it across neck of funnel until the small quantity of  $\text{CCl}_4$  on surface of aqueous phase has evaporated.

#### FINAL EXTRACTION

(Extraction of Zn in presence of carbamate reagent.)

Pipet 50 ml of Soln B and 10 ml of dithizone reagent into 50 ml of 0.02 *N* HCl soln containing the Zn. Shake for 1 min, then allow phases to separate. Flush out stopcock and stem of funnel with ca 1 ml of the  $\text{CCl}_4$  extract, then collect remainder in test tube. Pipet 5 ml of the extract into 25 ml volumetric flask, dilute to mark with clear  $\text{CCl}_4$ , and then determine % light transmission of diluted soln with a photoelectric colorimeter, equipped with a Sextant Green (Corning No. 401) filter, or equivalent.

In order to find correction to be applied for Zn present in reagents, run a blank with each series of Zn determinations exactly as done in case of the plant material, using as starting point an empty Pt dish that is placed in the muffle with the samples of plant material.

Zn on standard curve (% light transmission obtained for unknown—Zn (% transmission of blank) = Zn present in aliquot taken. From this figure calculate quantity of Zn in sample. (As many as 12 determinations may conveniently be carried simultaneously thru this procedure.)

#### STANDARD CURVE

Obtain data for standard curve by determining % transmission values for each of a series of solns containing known quantities of Zn. To prepare these Zn solns, place 0, 5, 10, 15, 20, 25, 30, and 35 ml of the standard Zn soln containing 10 gamma of Zn per ml in 100 ml volumetric flasks. To each flask add 1 drop of methyl red indicator, then normal  $\text{NH}_4\text{OH}$  until neutral, then 4 ml of normal HCl, and make to volume. Proceed exactly as directed for the ash solns, beginning with the first extraction, and using 10 ml aliquots of each of Zn solns. (The 10 ml aliquots contain 0, 5, 10, 15, 20, 25, 30, and 35 gamma of Zn, respectively.) Construct the standard curve by plotting gamma of Zn against % light transmission on semi-log paper.

### XIII. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

### XIV. MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS

No additions, deletions, or other changes.

## XV. WINES

The tentative method for the determination of gum and dextrin in wine, (35, p. 170) was deleted.

## XVI. DISTILLED LIQUORS

(1) The following method for the determination of tannins was adopted as tentative:

## TANNIN

## REAGENTS

(a) *Folin-Denis reagent*.—To 750 ml of  $H_2O$  add 100 g of sodium tungstate, 20 g of phosphomolybdic acid, and 50 ml of 85% phosphoric acid. Reflux for 2 hours, cool, and dilute to 1 liter.

(b) *Saturated sodium carbonate soln*.—For each 100 ml of  $H_2O$  at 70–80° add 35 g of anhydrous  $Na_2CO_3$ , dissolve, and allow to cool overnight. Feed the supersaturated soln with a crystal of  $Na_2CO_3$ , and after crystallization filter thru glass wool.

(c) *Standard tannic acid soln*.—Dissolve exactly 100 mg of tannic acid in 1 liter of  $H_2O$ . Prepare a fresh soln for each determination.

## DETERMINATION

Place 0.25–1.00 ml of whiskey in Nessler tube containing ca 90 ml of  $H_2O$ . Add 1.0 ml of the Folin-Denis reagent and make up to mark with  $H_2O$ . Then add 5.0 ml of the  $Na_2CO_3$  soln and shake well. After 10–15 min. compare the blue color developed with standards made in the same way at the same time containing 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 1.8, 2.0, and 2.4 ml of the standard tannic acid soln.

(2) The method for the determination of benzaldehyde (p. 184, 63, 64) was adopted as official (final action).

(3) The method for the determination of volatile esters in cordials (p. 183, 60) was adopted as official (final action).

(4) The method for the determination of gamma-undecalactone (qualitative), (p. 183, 61) was adopted as official (final action).

(5) The method for the determination of total solids from refractive index of dealcoholized sample (p. 181, 44(c)) was adopted as official (final action).

## XVII. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or other changes.

## XVIII. COFFEE AND TEA

No additions, deletions, or other changes.

## XIX. CACAO BEAN AND ITS PRODUCTS

The following method for the determination of the chocolate constituents of certain products was adopted as tentative:

## CHOCOLATE CONSTITUENTS

(Applicable to sweet chocolate, with or without small quantities of added dairy ingredients; milk chocolate; mixed milk chocolate; skim milk chocolate; buttermilk

chocolate; cocoa and fat, other than cacao fat, coatings; and chocolate and fat, other than cacao fat, coatings.)

Extract 25–50 g (50 g if light color, indicating low liquor) of sample as directed in 27 and 28, pp. 206–7, except to use in the first aqueous extraction 200 ml of  $H_2O$  or 1%  $Na_2C_2O_4$  soln, as the case may be.

With aid of small portions of ether (45, 20, 15 ml, etc.) transfer residue resulting from ether, alcohol, and aqueous extractions to tared aluminum dish provided with close-fitting cover. Use small quantity of acetone and a policeman to transfer any material that sticks to bottle. Evaporate liquid and dry residue in oven at  $100^\circ$ . Cover dish, cool in desiccator, and weigh.

To obtain the weight of fat-free dry cacao mass, multiply weight of residue by factor 1.43. To obtain weight of chocolate liquor, multiply weight of fat-free dry cacao mass by the factor 2.062. (This factor presupposes a standard requirement of 50% fat in chocolate liquor and assumes a moisture content of 1.5%.)

## XX. CEREAL FOODS

(1) The method for the determination of H-ion concentration (p. 213, 14) was adopted as official (final action).

(2) The methods for the determination of moisture, ash, protein, fat, and crude fiber in flour (p. 211, 2; 212, 5; 213, 10, 11; 213, 12) were adopted as tentative for the analysis of rice and barley products.

(3) The method presented on p. 143 for the determination of unsaponifiable matter in noodles and the farinaceous ingredients of noodles was adopted as official (first action).

(4) The method presented on p. 144 for the determination of sterols in noodles and the farinaceous ingredients of noodles was adopted as official (first action).

## XXI. COLORING MATTERS IN FOODS

No additions, deletions, or other changes.

## XXII. DAIRY PRODUCTS

(1) The following method for the determination of casein in malted milk and chocolate malted milk was adopted as tentative:

### CASEIN IN MALTED MILK AND CHOCOLATE MALTED MILK

Place a 10 g sample in centrifuge bottle of 250 ml (or larger) capacity and extract with two 100 ml portions of petroleum benzin by shaking until uniform, centrifuging, and decanting supernatant layer. To dry residue add exactly 200 ml of 3%  $Na_2C_2O_4$  soln. Shake occasionally over a 4 hour period. Centrifuge for 15 min. at high speed (1800 r.p.m. if No. 1 Sb bottle is used). Pipet 50 ml (100 ml for chocolate malted milk product) of supernatant liquid into 250 ml beaker. Add 50 ml of paper pulp soln (1 filter paper) and 2 ml of glacial acetic acid dropwise with constant stirring. Set beaker in warm  $H_2O$  ( $45$ – $50^\circ$ ) and let stand 15 min. Cool to room temp. and filter with moderate suction thru 7 cm Büchner funnel, previously fitted with No. 589 white ribbon paper and overlaid with a layer of paper pulp. Wash precipitate 2 or 3 times with cold  $H_2O$ . (Filtrate should be clear, or nearly so.) If first portions of filtrate are not clear, repeat filtration and complete the washing of precipitate. Determine  $N$  in washed precipitate and filter paper as directed in II, 25, and multiply by 6.38 to obtain equivalent of casein. Correct result for blank on reagents and paper pulp.

(2) The following rapid laboratory phosphatase test for pasteurization of milk, developed by the New York City Department of Health, was adopted as tentative:

#### RAPID (LABORATORY) PHOSPHATASE TEST FOR PASTEURIZATION<sup>1</sup>

##### COLLECTION OF SAMPLE

Proceed as directed under 1, except that if it is necessary to add a preservative only borax in the amount of 0.8 g per 100 ml of milk may be used. If samples sour, neutralize to pH 6.6 with  $\text{Na}_2\text{CO}_3$  before proceeding with analysis.

##### REAGENTS

(a) *Borate buffer*.—Dissolve 28.427 g of sodium borate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) in 900 ml of  $\text{H}_2\text{O}$ . Add 3.27 g of  $\text{NaOH}$  (81.75 ml normal  $\text{NaOH}$  soln) and dilute to 1 liter.

(b) *Buffer substrate*.—Dissolve 0.5 g of *crystalline* disodium phenyl phosphate in 5 ml of  $\text{H}_2\text{O}$  in a small (10×100 mm) test tube. Add 0.5 ml of borate buffer. Shake well and add 1/25 ml of the phenol reagent (or add 2 drops from a dropper delivering 50 drops per ml of the phenol reagent). Shake well. Allow 5 min. for color development. Extract indophenol by shaking with 2 ml of neutral *n*-butyl alcohol. Allow to stand until alcohol has separated completely. Remove supernatant alcohol layer with a pipet or medicine dropper and discard. Dilute remainder with 100 ml of borate buffer and sufficient  $\text{H}_2\text{O}$  to make 1 liter. (This buffer substrate is phenol-free.) Store under refrigeration. Because of possible decomposition, prepare quantities of this reagent sufficient for immediate needs only. The pH of this soln is ca 9.6 (blue to thymolphthalein soln—0.04% in 50% ethyl alcohol). Avoid intimate contact of soln with rubber. (A darkening indicates decomposition.)

(c) *Gibbs phenol reagent*.—Dissolve 40 mg of 2,6 dibromoquinone-chloroimide in 10 ml of methyl or 95% ethyl alcohol. Keep reagent tightly stoppered and under refrigeration.

(d) *Lead acetate soln*.—Dissolve 50 g of lead acetate [ $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ ] in 100 ml of  $\text{H}_2\text{O}$ .

(e) *Sodium pyrophosphate soln*.—Dissolve 10 g of sodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) in 100 ml of  $\text{H}_2\text{O}$ .

##### PERMANENT PHENOL STANDARDS

##### 1.—Acid Series:

(a) *Color soln, red*.—0.5 *N* cobalt chloride ( $\text{CoCl}_2$ ) in 1%  $\text{HCl}$  (59.59 g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  per liter).

(b) *Color soln, blue*.—30% copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in 1%  $\text{HCl}$  (300 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  per liter).

(c) *Color soln, yellow*.—0.5 *N* [M/6] ferric chloride ( $\text{FeCl}_3$ ) in 1%  $\text{HCl}$  (45.05 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  per liter).

Combine quantities indicated in table following and dilute to 5 ml with  $\text{H}_2\text{O}$ .

##### COLOR SOLUTIONS

Units	Blue ml	Red ml	Yellow ml
1	0.2	0.35	0.5
2	0.5	0.6	0.55
3.5	0.5	0.5	0.5
5	1.0	0.75	0.5
7.5	1.5	0.75	0.5
10	2.0	1.0	0.25

<sup>1</sup> References.—*J. Dairy Sci.*, 21, 21 (1938); *J. Milk Tech.*, 1, 85 (1938); Am. Public Health Assoc. and Assoc. Official Agr. Chem., Standard Methods for the Examination of Dairy Products, 7th ed., 1939, p. 174; *This Journal*, Report of F. W. Gilcreas, Associate Referee on Tests for Pasteurization of Dairy Products, to Annual Meeting, 1940 (to be published).

The following ammoniacal color standards supplement the acid series above by affording a more extensive range.

2.—*Ammoniacal Series:*

(a) *Color soln, red.*—Dissolve 1.8 g of roseo (aquopentamine) cobaltic chloride  $[\text{Co}(\text{NH}_3)_5 \cdot \text{H}_2\text{O}]\text{Cl}_2$  per liter of 2.8%  $\text{NH}_4\text{OH}$ .

(b) *Color soln, blue.*—6.24 g of copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) per liter of 2.8%  $\text{NH}_4\text{OH}$ .

(c) *Color soln, yellow.*—Dissolve 0.84 g of ammonium chromate  $[(\text{NH}_4)_2\text{CrO}_4]$  per liter of 2.8%  $\text{NH}_4\text{OH}$ .

Combine quantities indicated in table following and dilute to 5 ml with  $\text{H}_2\text{O}$ .

COLOR SOLUTIONS

Units	Blue <i>ml</i>	Red <i>ml</i>	Yellow <i>ml</i>
1	0.25	0.5	0.4
2	0.5	0.75	0.5
3.5	0.8	1.0	0.7
5	1.0	0.6	0.55
7.5	1.25	0.75	0.75
10	1.5	0.25	0.5
15	2.5		0.5
20	2.75		0.5
25	3.0		0.5
50	3.75		0.5
100	4.0		0.5
500	4.5		0.5

These color standards are suitable for use in natural or artificial light provided an opal double-fluxed glass or sheet of Plastacele No. C-1605 HH, 0.015" thick is used as a light filter. Use for color standards test tubes similar to those in which each test is conducted.

DETERMINATION

Transfer 1 ml of the milk to be tested to Pyrex test tube (15 mm  $\times$  125 mm) and add 10 ml of the buffer substrate. Mix thoroly. Warm to ca 40°, and place in incubator or water bath for 1 hour at 37–45° (41° preferred). After incubation, place tube in boiling  $\text{H}_2\text{O}$  for 5 min. Cool in ice  $\text{H}_2\text{O}$ . Add 0.1 ml of the Pb acetate soln. Shake immediately and well. (Proteins will coagulate and separate sharply. In some instances it may be necessary to add an additional 0.05 ml of Pb acetate.) Filter. To 5 ml of filtrate in a test tube (6  $\times$  5/8"), add 0.5 ml of the borate buffer. (Addition of a few drops of the pyrophosphate soln will clarify filtrate turbidity, if any.) Add 0.04 ml of the phenol reagent (2 drops from recommended dropper). Mix thoroly by rotating tube. After 15 min., estimate color by comparison with permanent color standards.

CONTROL TEST

(To check deterioration of reagents and presence of interfering substances in sample.)

To 5 ml of the buffer substrate, add 1/25 ml of the phenol reagent. (Development of blue color in 15 min. indicates extent of substrate decomposition.) Or, incubate 10 ml of buffer substrate with 1 ml of a boiled milk sample. Proceed as directed under Determination. (Indophenol development indicates extent of substrate decomposition.) To 9 ml of  $\text{H}_2\text{O}$  add 1 ml of the borate buffer and 1 ml of milk sample. Mix well, place in boiling  $\text{H}_2\text{O}$  for 5 min., and follow through as directed



under Determination. Development of blue color indicates extent of indophenol color due to interfering substances in milk sample.

#### INTERPRETATION

A phenol value of 2 units or less generally indicates milk heated to 143° F. for 30 min. Commercially pasteurized milk usually yields 0 to 1 unit because of added time exposure incurred by operating variations in preheating, filling, and emptying of tanks, etc. A value of 2 units or greater in commercially pasteurized milk indicates inadequate heat treatment.

(3) The specifications for filter paper given in the tentative phosphatase test for pasteurization of milk (p. 281, 36(d)) were changed to read as follows:

*Filter paper.*—Must be free from phenol and other interfering substances (Whatman No. 40 and Eaton & Dikeman "New Filt" Nos. 1 and 3 have been found satisfactory).

(4) The following method for the determination of total solids in plain ice cream was adopted as tentative:

#### TOTAL SOLIDS IN PLAIN ICE CREAM

Proceed as directed in XXII, 8, but without sand, using 1-2 gram sample. The sample may be weighed by means of a short, bent, 2 ml measuring pipet. Consider as total solids the residue obtained after heating for 4 hours.

(5) The tentative method for the determination of mold mycelia in butter (p. 300, 108, 109) was adopted as official (first action).

### XXIII. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

### XXIV. FISH AND OTHER MARINE PRODUCTS

No additions, deletions, or other changes.

### XXV. FLAVORING EXTRACTS

(1) The tentative method for the determination of  $\beta$ -ionone (p. 332, 68) was adopted as official (final action).

(2) The tentative method for the determination of  $\beta$ -ionone in raspberry flavors (pp. 332-3, 69, 70) was adopted as official (final action).

(3) The following directions for a more permanent standard vanillin solution were added to the official colorimetric method for the determination of vanillin (p. 321, 6):

*Stock soln of vanillin.*—Dissolve 1 g of vanillin in 250 ml of alcohol and dilute to 1 liter with H<sub>2</sub>O.

*Standard vanillin soln.*—Dilute 10 ml of the stock soln of vanillin to 100 ml with H<sub>2</sub>O. Use only freshly prepared solns.

### XXVI. FRUITS AND FRUIT PRODUCTS

The tentative colorimetric method for the determination of phosphoric acid (p. 347, 39-41) was adopted as official (first action).

## XXVII. GRAIN AND STOCK FEEDS

(1) The following precautionary notes were added to the method for the determination of grit in poultry and similar foods (p. 365, 46):

If sample contains salt remove from grit by washing with  $H_2O$ . Identify bone in grit by charring. If pelleted feeds or feeds containing molasses are being examined, disintegrate in cold  $H_2O$  and dry with alcohol or ether.

(2) The following method for the determination of ammoniacal and urea nitrogen was adopted as tentative:

## UREA AND AMMONIACAL NITROGEN

## REAGENTS

(a) *Standard acid*.—See II, 19(a) or (b).

(b) *Standard alkali*.—See II, 19(c).

(c) *Indicator*.—See II, 19(h) or (i).

(d) *Diglycol stearate soln*.—Dissolve 20 g of diglycol stearate Tech. in 1 liter of equal parts of benzol and ethyl alcohol (to prevent frothing).

(e) *Urease soln*.—Prepare fresh soln by dissolving standardized urease in  $H_2O$  so that each 10 ml of neutralized soln will convert the nitrogen of at least 0.1 g of pure urea. *Suggested standardization procedure*.—To determine alkalinity of commercial urease preparation dissolve 0.1 g in 50 ml of  $H_2O$  and titrate with 0.1 N HCl, using methyl red indicator. Add this quantity of 0.1 N HCl to each 0.1 g of urease in preparing the urease soln. To determine the enzyme activity prepare ca 50 ml of a neutralized 1% soln. Add different quantities of soln to 0.1 g samples of pure urea and follow with the enzyme digestion and distillation as directed in the determination. Calculate activity of the urease preparation from the quantity of this urease soln that converted the urea, thereby permitting complete recovery of the nitrogen by distillation.

## DETERMINATION

Place 2 g of sample in an 800 ml Kjeldahl digestion flask with ca 250 ml of  $H_2O$ . Add 10 ml of the urease soln, stopper tightly, and let stand at room temp. 1 hour or at 40° for 20 min. Cool at room temp. if necessary. Use more urease soln if feed contains more than 5% urea (ca 12% protein equivalent). Rinse stopper and neck with a few ml of  $H_2O$ . Add 2 g or more of MgO (heavy type), and 2 ml of the diglycol stearate soln, and connect flask with condenser by means of Kjeldahl connecting bulb. Distil 100 ml of the liquid into a measured quantity of the standard acid, and titrate with the standard alkali, using cochineal or methyl red indicator.

(3) The heading of the tentative method for the determination of carotene (p. 369, 61) was changed to read, "Crude Carotene in Hays and Dried Plants—Tentative."

(4) The method for the determination of manganese (p. 369, 59, 60) was changed to read as follows and adopted as official (first action):

## ACID-SOLUBLE MANGANESE

Ash a weighed sample, 5–15 g, at dull red heat in porcelain dish. When cool, add 5 ml of  $H_2SO_4$  and 5 ml of  $HNO_3$  to the ash in the dish or to the ash transferred to a beaker with 20–30 ml of  $H_2O$ . Evaporate to white fumes. If carbon is not completely destroyed, add further portions of  $HNO_3$ , boiling after each addition. Cool slightly, transfer to 50 or 100 ml volumetric flask, and add a volume of  $H_3PO_4$  soln (8 ml of 85%  $H_3PO_4$  + 92 ml of  $H_2O$ ) equal to  $\frac{1}{2}$  the volume of the flask (25–50 ml). Cool,

make to volume, mix, and filter or let stand until clear. If a 50 ml flask was used, pipet 25 ml of clear soln into a beaker or 50 or 100 ml volumetric flask and add 15 ml of  $H_2O$ . If a 100 ml flask was used, pipet 50 ml into a beaker or a 100 ml flask and add 30 ml of  $H_2O$ . Heat nearly to the boiling point, and with stirring or swirling add 0.3 g of  $KIO_4$  for each mg of Mn present. Compare with the standard  $KMnO_4$  soln in a colorimeter. Calculate p.p.m. of Mn in the sample.

(5) The following photoelectric colorimeter method for the determination of carotene was adopted as tentative:

#### CAROTENE

##### *Photoelectric Colorimetric Method*

#### REAGENTS

(a) *Purified solns of carotene*.—Dissolve 0.1 g of crystalline carotene in as little  $CHCl_3$  (2–4 ml) as possible. Add 20 ml of absolute methanol and filter. Wash the precipitated carotene once with 2 or 3 ml of methanol, and dry in vacuo over  $H_2SO_4$ . Weigh 20 mg of purified carotene, dissolve in 2 ml of  $CHCl_3$ , and dilute to exactly 100 ml with petroleum benzin.

(b) *Potassium dichromate*.—0.02%. Weigh accurately 0.2000 g of C.P.  $K_2Cr_2O_7$ , dissolve in ca 100 ml of  $H_2O$ , and dilute to 1000 ml.

#### CALIBRATION

Dilute 5 ml of the stock soln to 500 ml with petroleum benzin (Soln B). Then dilute 40 ml of Soln B to 50 ml (Soln C), 30 ml of Soln B to 50 ml (Soln D), 20 ml of Soln B to 50 ml (Soln E), 10 ml of Soln B to 50 ml (Soln F), and 5 ml of Soln B to 50 ml (Soln G). Read Solns B, C, D, E, F, and G, which contain 2.0, 1.6, 1.2, 0.8, 0.4, and 0.2 p.p.m. of carotene, respectively, in the photoelectric colorimeter. Plot colorimeter readings against concentration of carotene on arithmetic graph paper. Use this curve for estimating carotene in solns. If the photoelectric colorimeter does not have the sensitivity required to give proper response to above concentrations of carotene, use 6 other concentrations of carotene with approximately same degree of difference as ones given.

Check colorimeter from time to time against a known concentration of the pure carotene or of the  $K_2Cr_2O_7$ . If the latter is used, always use same light filters in the colorimeter, as the carotene equivalent of  $K_2Cr_2O_7$  is not the same for all light filters. If colorimeter is more than 5% off, recalibrate.

#### DETERMINATION

Place the carotene solns in the colorimeter and read the color. From the colorimeter reading, locate on the calibration curve the corresponding concentration of carotene and calculate carotene in solution to 0.1 p.p.m.

### XXVIII. MEAT AND MEAT PRODUCTS

No additions, deletions, or other changes.

### XXIX. METALS IN FOODS

The following changes were made in the official rapid method for the determination of lead (pp. 407–9, 30–32):

- 31(a) (1) In line 5, 9.82 mg was changed to 18.2 mg.
- (2) In lines 10 and 11, 0.027 grain/lb. was changed to 0.050 grain/lb.
- (3) In line 12, 0.003 grain/lb. was changed to 0.005 grain/lb.

(4) In line 13, 0.001 grain/lb. was changed to 0.002 grain/lb.

(5) The table under 31(a) was changed to read as follows:

Grain/lb.	Standard	Blank
	<i>ml</i>	<i>ml</i>
0.000	0.0	10.0
0.005	1.0	9.0
0.010	2.0	8.0
0.015	3.0	7.0
0.020	4.0	6.0
0.025	5.0	5.0
0.030	6.0	4.0
0.035	7.0	3.0
0.040	8.0	2.0
0.045	9.0	1.0
0.050	10.0	0.0

(6) In first line under table the reagent, 13(p), p. 397, was changed to read 250 ml of  $\text{NH}_4\text{OH}$  instead of 500 ml of  $\text{NH}_4\text{OH}$ .

(7) In the second line under the table, 25 ml was changed to 20 ml.

(8) In the seventh line under the table, 0.015–0.025 was changed to 0.030–0.050.

31(b) (9) In lines 1, 16, and 19, 20 ml was changed to 10 ml.

(10) In lines 3 and 4, 20 ml was changed to 30 ml.

(11) In line 16, 0.027 was changed to 0.050.

(12) In line 17, 10 ml was changed to 5 ml.

32 (13) In line 3, 10 ml was changed to 5 ml.

(14) The table was changed to read as follows:

Grain/lb.	0	0.010	0.020	0.030	0.040	0.050
Standard (ml)	0	1.0	2.0	3.0	4.0	5.0
Blank (ml)	10	9.0	8.0	7.0	6.0	5.0

(15) In line 7, the ammonia-cyanide-citrate solution, 13(p) was changed as noted previously.

(16) In line 8, 20 ml was changed to 25 ml.

32(b) (17) In line 2, 20 was changed to 10 ml.

(18) In line 3, the ammonia-cyanide-citrate solution, 13(p), was changed as noted previously, and 20 ml was changed to 25 ml.

(19) In line 6, 10 ml was changed to 5 ml.

### XXX. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

### XXXI. OILS, FATS, AND WAXES

No additions, deletions, or other changes.

### XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

The following qualitative test for the determination of saccharin in non-alcoholic beverages, semi-solid preparations, and baked goods was adopted as tentative:

**SACCHARIN**

(Applicable to non-alcoholic beverages, semi-solid preparations, and baked goods.)

**REAGENTS**

(a) *Ethereal solvent*.—Mix equal volumes of ethyl ether and petroleum benzin (b.p. 30–60°).

(b) *Phenol-sulfuric acid*.—Dissolve pure colorless crystalline phenol in an equal weight of  $\text{H}_2\text{SO}_4$ .

(c) *Petroleum benzin*.—(b.p. 30–60°).

(d) *Sea sand*.—Washed and ignited.

**PREPARATION OF SAMPLE**

(a) *Non-alcoholic beverages*.—Add 3 ml of  $\text{HCl}$  to 25 ml of sample contained in separatory funnel. If vanillin is present, remove it by extracting with several portions of petroleum benzin. Discard the petroleum benzin. Extract with 50, 25, and 25 ml of the ethereal solvent. Wash the combined ethereal extracts once with 5 ml of  $\text{H}_2\text{O}$ , remove major portion of solvent, transfer to 30 ml beaker, and allow to dry at room temp.

(b) *Semi-solid preparations*.—Transfer 25 g of sample to 100 ml volumetric flask with small quantity of hot  $\text{H}_2\text{O}$  and add sufficient boiling  $\text{H}_2\text{O}$  to make ca 75 ml. Allow mixture to stand for an hour, shaking occasionally. Then add 3 ml of glacial acetic acid, mix thoroly, add a slight excess (5 ml) of 20% neutral  $\text{Pb}$  acetate soln, dilute to mark with cold  $\text{H}_2\text{O}$ , mix, allow to stand for 20 min., and filter. Transfer 60 ml, or more of the filtrate, to separatory funnel and proceed as directed under (a).

(c) *Baked goods*.—Grind 25 g of sample, mix thoroly with 50 g of the sea sand, and extract with petroleum benzin in a Soxhlet until approximately fat free (1–2 hours). Transfer extracted mass to 300 ml Erlenmeyer flask, add 100 ml of alcohol, and reflux on boiling water bath for 30 min., shaking frequently. Filter thru a Büchner containing a No. 2, 7 cm filter paper wet with alcohol. Transfer the alcoholic filtrate to 100 ml beaker, evaporate to  $\frac{1}{2}$  volume, add 50 ml of  $\text{H}_2\text{O}$  and sufficient 10%  $\text{Na}_2\text{CO}_3$  soln to make alkaline, and evaporate to 50 ml. Transfer aqueous soln to separatory funnel and proceed as directed under (a).

**DETERMINATION**

Add 5 ml of the phenol- $\text{H}_2\text{SO}_4$  reagent to the residue remaining after the evaporation of solvent and heat for 2 hours at 135–140°. Cool, dissolve in small quantity of hot  $\text{H}_2\text{O}$ , and pour into ca 250 ml of  $\text{H}_2\text{O}$ . Add small quantity of filter-cel, allow to stand 3 hours or overnight, and filter. Make alkaline with 10%  $\text{NaOH}$  soln and dilute to 500 ml. A magenta or reddish purple color develops if saccharin is present. A yellow, buff, or pale salmon shade is significant.

**XXXIII. SPICES AND OTHER CONDIMENTS**

(1) The following methods for the determination of soluble and insoluble phosphoric acid in vinegars were adopted as tentative:

**SOLUBLE PHOSPHORIC ACID**

Proceed as directed under II, 9 or 12, or XXVI, 41, using soln obtained under 62. If either volumetric or colorimetric method is used, standardize with a sample of known phosphate content. Express results as mg of  $\text{P}_2\text{O}_5$  per 100 ml of vinegar.

**INSOLUBLE PHOSPHORIC ACID**

Dissolve water-insoluble ash, 61, in ca 50 ml of boiling  $\text{HNO}_3$  (1+8). (Use 25 ml of  $\text{H}_2\text{SO}_4$  (1+9) if colorimetric method is used) and proceed as directed under

II, 9 or 12, or XXVI, 41. If either volumetric or colorimetric method is used, standardize with a sample of known phosphate content. Express result as mg of  $P_2O_5$  per 100 ml of vinegar.

(2) The official methods for the determination of soluble and insoluble phosphoric acid in vinegars (p. 478, 63, 64) were dropped (first action).

(3) The following method for the determination of total phosphoric acid in vinegars was adopted as tentative:

#### TOTAL PHOSPHORIC ACID

Dissolve ash, 60, or both soluble and insoluble ash, 61, in ca 50 ml of boiling  $HNO_3$  (1+8). Use 25 ml of  $H_2SO_4$  (1+9) if colorimetric method is used and proceed as directed under II, 9 or 12, or XXVI, 41. If either volumetric or colorimetric method is used, standardize with a sample of known phosphate content. Express result as mg of  $P_2O_5$  per 100 ml of vinegar. If desired, digest vinegar as directed in XXVI, 40, instead of using ash from 60.

(4) The tentative method for preparation of sample of mayonnaise and salad dressing (p. 475, 44) was changed to read as follows:

#### PREPARATION OF SAMPLE

Before removing any portion of sample for analysis, transfer to suitable container, such as glass fruit jar of larger capacity than volume of sample, mix until homogeneous with a spatula (2-3 min. should be sufficient). Repeat mixing before each subsequent portion is removed for analysis if sample has stood for any appreciable length of time. For the various determinations, take approximately the quantity directed and weigh. (A light 100 ml flask fitted with a straight glass tube and over-size rubber bulb makes a suitable weighing bottle.)

(5) The tentative method for the determination of total fat in mayonnaise and salad dressing (p. 476, 52) was modified to provide for a 1 gram sample instead of a 2 gram sample.

(6) The tentative methods for the determination of sugars and for the identification of the oil in mayonnaise and salad dressing (pp. 475, 6, and 7, 46-48, 54) were adopted as official (first action).

(7) The following method for direct determination of moisture in spices was adopted as tentative:

#### MOISTURE

Clean the distilling tube receiver and condenser described under XXVII, 3, with  $Cr_2O_7-H_2SO_4$  mixture, rinse thoroly with  $H_2O$ , then with approximately 0.5 N alcoholic KOH soln, and allow apparatus to drain for 10 min. Remove connecting stopper from condenser before cleaning, so that it remains dry. Place 40 g of spice in the distilling flask and determine moisture as directed in 4, XXVII.

(8) The official method for the determination of ash in spices (p. 468, 3) was dropped (first action).

(9) The following method for the determination of ash in spices was adopted as tentative:

#### ASH

Weigh accurately ca 2 g of sample in flat-bottomed dish, preferably of Pt. Place dish in entrance of open muffle so that sample fumes off without catching fire. Place

dish in muffle kept at 550° for 30 min., break up ash with several drops of H<sub>2</sub>O, evaporate carefully to dryness, and heat in muffle for 30 min. If previous wetting showed ash to be free from C, remove dish to desiccator containing fresh efficient desiccant (H<sub>2</sub>SO<sub>4</sub> and anhydrous Mg(ClO<sub>4</sub>)<sub>2</sub> are satisfactory), allow to cool to room temp. and weigh soon. If first wetting showed C, repeat wetting and heating until no specks of C are visible, then heat for 30 min. after disappearance of C. If C persists, leach ash with hot H<sub>2</sub>O, filter thru quantitative filter paper, wash paper thoroly, transfer paper and contents to ashing dish, dry, and ignite in muffle at 550° until ash is white. Cool dish, add filtrate, evaporate to dryness on steam bath, and heat in muffle for 30 min. Cool, and weigh as directed previously.

*Nutmeg, mace, ginger, and cloves.*—Heat at 600° without appreciable loss of ash constituents.

*Ground mustard or mustard flour.*—Ignite as directed previously and heat for 30 min. at 550°. Leach ash with hot H<sub>2</sub>O, filter, and wash thoroly. Transfer filter paper and contents to ashing dish, dry, and heat in muffle for 30 min. Remove dish, allow to cool, add 5–10 drops of HNO<sub>3</sub>, evaporate to dryness, and heat in muffle 30 min. Repeat HNO<sub>3</sub> and heating treatment until residue is white. Add filtrate, evaporate, to dryness, and heat in muffle for 30 min. Cool, and weigh as directed previously.

(10) The method for determining the iodine number of paprika oil (p. 472, 27) was adopted as official (final action).

#### XXXIV. SUGARS AND SUGAR PRODUCTS

No additions, deletions, or other changes.

#### XXXV. VEGETABLES AND VEGETABLE PRODUCTS

(1) The tentative method for the determination of total solids in tomato products (p. 520, 18) was adopted as official (first action). The explanatory note in parentheses under the caption was deleted.

(2) The official method for the determination of chlorides in tomato juice (*This Journal*, 20, 78; 21, 90; 22, 88) was dropped (final action).

#### XXXVI. VITAMINS

(1) The method submitted by the associate referee for the determination of Vitamin B<sub>1</sub> was adopted as tentative (see p. 150).

(2) The following assay for the determination of Vitamin K was adopted as tentative:

##### BIOLOGICAL ASSAY FOR VITAMIN K

(This assay is a comparison under specified conditions of the antihemorrhagic potency of any product with a chemically pure standard antihemorrhagic compound in controlling the blood prothrombin level of the blood of chicks.)

Place 1- or 2-day-old chicks that have not been fed in metal, wire-meshed-floored, electrically heated, battery brooders. Maintain temp. within battery compartments at 85–90° F. Have food and water receptacles outside the compartment, but available to chick through apertures that permit only the head of chick to pass; provide fresh water daily in clean troughs. To prevent bacterial synthesis discard any portion of ration that becomes wet, and use every precaution to prevent access of chicks to droppings. Discard any unused portion of ration every 4 days or oftener.

Provide all chicks with the following basal ration, which must be finely ground and uniformly mixed.

	<i>per cent</i>
Sardine (Pilchard) meal <sup>(1)</sup> .....	17.5
Dried brewer's yeast <sup>(1)</sup> .....	7.5
Ground polished rice.....	72.5
Cod-liver oil, U.S.P.....	1.0
Calcium carbonate.....	0.5
Sodium chloride <sup>(2)</sup> .....	1.0

(1) Prepare by extracting continuously for 24 hours with ethyl ether.

(2) Add sufficient Mn in form of sulfate or carbonate to make 0.5% of the NaCl.

Maintain chicks on basal ration until clotting time of blood samples is 15 min. or more, as determined on 5% of chicks (usually 10-14 days). The following procedure may be used in determining clotting time:

A few drops of blood are withdrawn by making a small clean cut in an exposed wing vein at the junction of ulna, radius, and humerus. The blood is put in small vials and placed in shaking device in thermostatically-controlled water bath, described later for the determination of prothrombin clotting time. The time interval from withdrawal of blood to formation of firm clot is measured.

When the chicks show the prescribed delayed clotting time divide them into uniform groups of not less than 12 each. Have at least 2 groups receiving different dosages of 2 methyl 1,4 naphthoquinone as a reference standard, and at least one group for each product to be assayed. Maintain 1 group of 12 chicks on the basal ration only to serve as a negative control group.

Administer the materials to be assayed and the standard orally as solns. Dissolve a weighed or measured quantity of the material to be administered in sufficient quantity of diluent so that desired daily dose is contained in 0.1 ml. If the material to be administered is soluble in H<sub>2</sub>O, use distilled H<sub>2</sub>O as diluent, and if it is fat-soluble use ethyl laurate. Administer a prescribed dose daily by mouth with tuberculin syringe or other equally suitable measuring device. In administering dose, open mouth of chick with pressure at corners of mouth, and permit soln to fall well down throat. Do not permit chicks to have access to food or H<sub>2</sub>O for one-half hour after receiving the vitamin K reference standard or assay products. Continue individual daily dose for 4 days at ca 24-hour intervals.

Approximately 24 hours after administration of last dose of vitamin K standard and assay products determine prothrombin time on all chicks. Prepare clotting agent according to following procedure:

Select the 4 or 5 chicks to be used for this preparation at time assay is begun and maintain them on a practical chick ration containing at least 5% of dried alfalfa. House chicks in a manner to prevent contamination of rations of those receiving the vitamin K standard or the assay product. Kill one of birds reserved for preparation of clotting agent by bleeding. Excise 10 g of breast muscle and grind with sand and 10 ml of 0.85% NaCl soln. Centrifuge mixture and filter thru very coarse paper. (The resulting liquid may be stored in refrigerator for several days but preferably a fresh tissue extract should be used for each group of tests.) Dilute extract to 200 ml with 0.85% NaCl soln and then mix 50 ml with an equal volume of 0.025 M CaCl<sub>2</sub> soln. (This mixture should clot blood of normal chicks reared on practical mash in 20-30 seconds when tested according to procedure described below. If it does not, the concentration of clotting agent should be altered until the prothrombin clotting time falls within this range. The blood of negative control chicks will usually fail to clot in less than 80 seconds.) Place 0.2 ml of 0.1 M Na oxalate soln in short and narrow tubes calibrated to 2 ml. Introduce 1.8 ml of blood from a chick into each tube. (The blood may be obtained conveniently by cutting off head of chick with scissors and directing the blood into tube with fingers.) Shake tube thoroly when blood



has been drawn to the 2 ml mark. Pipet 0.1 ml samples of the oxalated blood into small, cylindrical, flat-bottomed vials 15×50 mm. Add 0.2 ml of the clotting agent and simultaneously start a stop-watch. Place vial in thermostatically-controlled water bath adjusted to 38.5–39.0°. Use a device to tilt vials at angle of ca 45° and back to a vertical position once per second. When bottom of vial is covered by a definite gelatinous film indicating clot formation, stop the watch. (Lapse of time indicated by watch is prothrombin time.) Duplicate procedure on blood from same chick until results do not vary by more than 2 seconds.

#### COMPUTATION OF RESULTS

Calculate mean prothrombin time for each group. Plot reciprocal of mean prothrombin time against logarithm of dosage of vitamin K expressed in micrograms for groups receiving the 2 methyl 1,4 naphthoquinone reference standard and connect points with straight line. Determine where reciprocal of mean prothrombin time for any group that received a product under assay intersects this line to determine logarithm of vitamin K activity equivalent. 1 microgram of reference standard = 1 unit of vitamin K activity.

(3) The following microbiological and fluorometric methods were adopted as tentative for the determination of riboflavin in yeast and dried skim milk:

#### RIBOFLAVIN IN YEAST AND DRIED SKIM MILK

##### *Microbiological Method*

(This method depends upon use of pure culture of *Lactobacillus Casei*  $\epsilon$ ; contamination with other organisms invalidates results.)

#### REAGENTS

(a) *Yeast extract*.—Dissolve 2 g of Difco yeast extract in H<sub>2</sub>O and dilute to 100 ml.

(b) *Agar*.—Difco Bacto or some equally pure agar.

(c) *Yeast supplement*.—Dissolve 50 g of Difco yeast extract in 250 ml of H<sub>2</sub>O. Add 75 g of basic Pb acetate (Horne's sugar reagent) dissolved in 250 ml of H<sub>2</sub>O. Centrifuge off precipitate, add NH<sub>4</sub>OH to filtrate to a pH of ca 10.0, and filter off precipitate. Add glacial acetic acid to filtrate until slightly acid, then pass in H<sub>2</sub>S until excess Pb is precipitated. Filter, and dilute filtrate to 500 ml. 1 ml of this soln = 100 mg of yeast extract. Add ca 5 ml of toluol to preserve soln.

(d) *Peptone, photolyzed*.—Dissolve 20 g of Difco Bacto peptone in 125 ml of H<sub>2</sub>O. Dissolve 10 g of NaOH in 125 ml of H<sub>2</sub>O. Mix two solns, place mixture in 9" crystallizing dish, and expose to light from 100-watt bulb with reflector at distance of ca 1 ft. for 6–10 hours. Then allow mixture to stand 18–14 hours (24 hours in all). Neutralize the NaOH with glacial acetic acid. Add 3.5 g of anhydrous Na acetate and dilute to 400 ml. Add ca 5 ml of toluol to preserve soln.

(e) *Potassium phosphate*.—Dissolve 25 g of K<sub>2</sub>HPO<sub>4</sub> and 25 g of KH<sub>2</sub>PO<sub>4</sub> in 250 ml of H<sub>2</sub>O.

(f) *Mixed salts*.—Dissolve 10 g of MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g of NaCl, 0.5 g of FeSO<sub>4</sub>·7H<sub>2</sub>O, and 0.5 g of MnSO<sub>4</sub>·4H<sub>2</sub>O in 250 ml of H<sub>2</sub>O.

(g) *Cystine soln*.—Dissolve 0.5 g in ca 400 ml of H<sub>2</sub>O containing ca 3 ml of HCl, and dilute to 500 ml. Add 5 ml of toluol.

(h) *Glucose*.—Use anhydrous reagent grade.

(i) *Pure culture of Lactobacillus casei*  $\epsilon$ .—This culture may be obtained from

Dr. J. V. Anzulovic, American Type Culture Collection, Georgetown University Medical School, 3900 Reservoir Rd., Washington, D. C. Ask for *Lactobacillus casei* No. 7469. The cost of one culture is \$2.00 plus 35¢ to cover cost of packing and postage. The cultures may be received free of charge from the associate referee.

(j) *Standard riboflavin solns.*—Store in dark bottles in refrigerator. Add 5 ml of toluol for preservation. Protect from light.

*Soln A.*—Weigh 50 mg of 100% Merck's synthetic riboflavin on an analytical balance and dilute to 1000 ml with 0.02 N acetic acid (12 g of glacial acetic acid to 1000 ml of H<sub>2</sub>O). (This soln is assumed to contain 50 micrograms of riboflavin per ml.)

*Soln B.*—By means of a pipet place 5 ml aliquot of Soln A into 250 ml volumetric flask and dilute (in the dark) to mark with 0.02 N acetic acid. (This soln is assumed to contain 1 microgram of riboflavin per ml.)

*Soln C.*—Pipet 10 ml of Soln B into 100 ml volumetric flask and dilute to mark with H<sub>2</sub>O. (This soln is assumed to contain 0.1 microgram per ml.) Discard soln after using.

(k) *Agar tube cultures (culture medium for Lactobacillus casei ε).*—Dissolve 0.5 g of the glucose in 100 ml of the yeast extract and add 1.5 g of the agar. Heat mixture in autoclave or in water bath until agar is dissolved. Dilute to 100 ml and mix well. Place 10 ml portions in test tubes and plug with cotton. Sterilize in autoclave at pressure of 15 lbs. for 15 min. Allow tubes to cool in upright position.

(l) *Liquid culture medium.*—Mix 100 ml of the photolyzed peptone, 100 ml of the cystine soln, 10 g of the glucose, 10 ml of the yeast supplement, 5 ml of the potassium phosphate, and 5 ml of the mixed salts.

Test the pH and adjust to 6.6–6.8 if necessary by small additions of 0.5% KOH or HCl (1+10). Use bromothymol blue as indicator. Dilute to 500 ml. If this medium is not used immediately, pipet 5 ml portions into test tubes, plug with cotton, and autoclave at 15 lbs. pressure for 15 min.

(m) *Sodium chloride soln.*—Dissolve 9 g of NaCl (pure) and make up to 1000 ml. Sterilize this soln when needed by placing 10 ml in test tubes and autoclaving at 15 lbs. pressure for 15 min.

#### PREPARATION OF INOCULUM

(1) *Stock cultures.*—Make stab cultures into 3 or more of the agar tubes from the original pure culture of *Lactobacillus casei ε*. Incubate at 37° for 24 hours and store in refrigerator until needed. Keep one of cultures (especially if future work is to be done) as a reserve stock culture and do not disturb it except to make new stock cultures from it at end of 1 month. (The other stock tubes are to be used for the analyses and are good for 1 month only. If work is to be done after that time, make new stock cultures from the reserve stock culture.) Discard cultures when 1 month old.

(2) *Sodium chloride inoculum.*—Pipet 5 ml of the liquid culture medium into 6 test tubes (16×150 mm to 20×150 mm). To each culture medium tube add 1.0 ml of Riboflavin Soln B and 4 ml of H<sub>2</sub>O (final volume 10 ml). Plug tubes with cotton and sterilize by autoclaving at 15 lbs. pressure for 15 min. Avoid exposing tubes to light at any time after riboflavin is added. After tubes are cool, inoculate 3 with a stab from one of stock cultures. Incubate 24 hours at 37°. (Three cultures are made in order to replace any lost ones.) Transfer 3 drops of the culture from one of tubes into each of remaining 3 tubes of liquid culture medium. Incubate 24–40 hours at 37°. Centrifuge out cells aseptically. Resuspend cells from one of tubes in 10 ml of sterile 0.9% soln of the NaCl. This culture is to be used as the inoculum and may be kept 5 days (store in refrigerator).

## PREPARATION OF SAMPLE

(a) *Yeast or other materials containing over 20 p.p.m. of riboflavin.*—To 2 g add 200 ml of  $H_2O$ , mix well, and autoclave at  $120^\circ$  for 15 min. Centrifuge off insoluble matter and wash twice with 20 ml of  $H_2O$ , using the centrifuge. Combine extracts and dilute to 1000 ml in a graduated flask. 1 ml of this extract = 2 mg of the sample. Avoid exposure to light at all times.

(b) *Skim milk or other samples containing 10–20 p.p.m. of riboflavin.*—To 3 g add 150 ml of 0.1 *N* HCl. Mix well and autoclave 15 min. at  $120^\circ$ . Adjust pH to 6.6–6.8 with 0.1 *N* NaOH (bromothymol blue as indicator) and dilute to 500 ml in graduated flask. Assay this suspension directly. 1 ml = 6 mg of sample. Avoid exposure to light.

## PROCEDURE

With each set of assays run known quantities of riboflavin. Use duplicate tubes, each containing 0.0, 0.05, 0.075, 0.1, 0.15, 0.2, and single tubes, each containing 0.3 and 0.5 microgram of riboflavin. With a microburet, measure 0.0, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, and 5.0 ml of the Standard Riboflavin Soln C into test tubes (specified above) and pipet in 5 ml of the liquid culture medium. Add enough  $H_2O$  to make final volume 10 ml.

Use duplicate tubes for the samples to be analyzed containing the equivalent of 0.5, 1, 2, 3, 4, and 5.0 mg. of yeast or 1.5, 3, 6, 9, 12, and 15 mg. of dried skim milk. Measure 0.25, 0.5, 1.0, 1.5, 2, and 2.5 ml of yeast extract or of the dried milk suspension into test tubes and pipet in 5 ml of the liquid culture medium. Add enough  $H_2O$  to make final volume 10 ml.

Plug tubes with cotton and sterilize in the autoclave at 15 lbs. pressure for 15 min. Allow to cool, and inoculate each with 1 drop of the 0.9% NaCl inoculum. Incubate at  $37^\circ$  for 3 days. Transfer contents to 125 ml Erlenmeyer flasks, wash tubes with 10–20 ml of  $H_2O$  and titrate from a microburet to a pH of 7.0 with 0.1 *N* NaOH, using bromothymol blue as indicator.

## CALCULATION OF RESULTS

Plot the micrograms of riboflavin against ml of 0.1 *N* NaOH used in the titration of the standard riboflavin. If the blank is 1 ml or over, the ingredients of the liquid culture contain riboflavin and the results should not be used. Then run the experiment again with reagents more rigorously freed from riboflavin.

From volume of 0.1 *N* NaOH used in titration for each level of yeast and of dried skim milk locate on graph corresponding micrograms of riboflavin. These values should be 0.05–0.25 micrograms, otherwise they are not reliable. If 3 of the values do not fall between this range repeat the analysis with different quantities calculated from results obtained. Calculate p.p.m. (micrograms per gram) for each quantity in range specified. (The results from each quantity should agree within 20% with the results from the other quantity.) Average the quantities in this range for the final result.

## Fluorometric Method

## REAGENTS

(a) *Sulfuric acid.*—Approximately 0.25 *N*. Dilute 5 ml of  $H_2SO_4$  to 800 ml with  $H_2O$ .

(b) *Tri-sodium phosphate soln.*—Dissolve 65 g of  $Na_3PO_4 \cdot 12H_2O$  in 1 liter of  $H_2O$ .

(c) *Acid-acetone soln.*—Add 3 volumes of commercial acetone to 1 volume of normal  $H_2SO_4$  (5 ml of  $H_2SO_4$  in 200 ml of  $H_2O$ ). (This reagent is used only if products contain casein.)

(d) *Sodium hydrosulfite soln.*—Dissolve 1 g of Na hydrosulfite ( $\text{Na}_2\text{S}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ ) and 1 g of  $\text{NaHCO}_3$  in 20 ml of ice-cold  $\text{H}_2\text{O}$  and keep in ice bath. (This soln is stable for ca 4 hours.)

(e) *Stannous chloride.*—Dissolve 10 g of  $\text{SnCl}_2$  in 25 ml of  $\text{HCl}$  and store in brown glass-stoppered bottle. For determination dilute 1 ml of above stock soln with 200 ml of  $\text{H}_2\text{O}$ . Make a fresh preparation of the dilute soln daily.

(f) *Standard riboflavin solns.*—Store in dark bottles in refrigerator. Rigorously protect from light.

(1) *Soln A.*—Weigh 20 mg of Merck's 100% pure synthetic riboflavin, dissolve, add a few drops of glacial acetic acid, and dilute to 500 ml with  $\text{H}_2\text{O}$ . This soln is assumed to contain 40 p.p.m. of riboflavin.

(2) *Soln B.*—Pipet 25 ml of Soln A into 100 ml flask and dilute to volume. This soln is assumed to contain 10 p.p.m. of riboflavin.

(g) *Standard quinine sulfate solns.*—Used to regulate intensity of activating light of a fluorometer. (They cannot be used for visual comparisons of riboflavin. Sodium fluorescein, or a standard glass cube may be substituted for quinine sulfate.)

(1) *Soln A.*—Weigh 40 mg of quinine sulfate, dissolve in ca 20 ml of 0.1  $N$   $\text{H}_2\text{SO}_4$ , and then dilute to 200 ml in graduated flask with the acid.

(2) *Soln B.*—Pipet 10 ml of Soln A into 100 ml graduated flask and make up to volume with 0.1  $N$   $\text{H}_2\text{SO}_4$ .

(3) *Soln C.*—Pipet 10 ml of Soln B into 1000 ml graduated flask and make up to volume with 0.1  $N$   $\text{H}_2\text{SO}_4$ . Soln C contains 0.2 p.p.m. of quinine sulfate.

#### CALIBRATION CURVE OF FLUOROMETER

(The directions given are for calibration of fluorometer manufactured by Pfaltz and Bauer, Inc., New York. Other types of instruments may be used. Such instruments may not have same sensitivity as the Pfaltz and Bauer instrument and therefore riboflavin solns of different concentrations may be needed.)

Pipet 20 ml of the standard riboflavin soln B (f) (2) into a 200 ml graduated flask and dilute to volume (Soln C). Dilute 40 ml of Soln C to 50 ml (Soln D). Dilute 10 ml of Soln B (f) (2) to 200 ml (Soln E). Dilute 40 ml of Soln E to 50 ml (Soln F), 30 ml of Soln E to 50 ml (Soln G), 20 ml of Soln E to 50 ml (Soln H), 10 ml of Soln E to 50 ml (Soln I), 5 ml of Soln E to 50 ml (Soln J), and 0.0 ml of Soln E to 50 ml (Blank). Soln C contains 1.0 p.p.m. riboflavin, Soln D 0.8, Soln E 0.5, Soln F 0.4, Soln G 0.3, Soln H 0.2, Soln I 0.1, and Soln J 0.05.

Place the quinine sulfate soln containing 0.2 p.p.m. (g) (3) in the cell of the fluorometer and adjust iris diaphragm of instrument to give galvanometer deflection of 65 mm. Remove the quinine sulfate and obtain the galvanometer deflections for riboflavin solutions C, D, E, F, G, H, I, J, and blank. Also obtain the galvanometer deflections for the above riboflavin solns for quinine sulfate deflections of 50.0, 33.0, and 18.2. Plot curve of concentration of riboflavin against galvanometer deflection for each deflection of quinine sulfate. Subtract reading for blank from each value. Place all curves on same graph. By above procedure a wide range of concentration of riboflavin can be read on the fluorometer.

#### DETERMINATION

##### *Procedure A—Yeast*

*For materials that do not contain casein.*—Avoid exposure to light as much as possible. Weigh 5 g into 300 ml Erlenmeyer flask and pipet in 50 ml of the 0.25  $N$   $\text{H}_2\text{SO}_4$ . Mix thoroly and break up the lumps. Boil gently under a reflux condenser for 1 hour. Allow sample to come to room temp. and remove from condenser. Bring the pH to 7.0–7.5 with the tri-sodium phosphate soln. Use phenol red or other suit-

able indicator. (30 ml. of the phosphate soln may be added before the soln is tested for pH.) Uses only 1 drop of soln for each pH test. Transfer soln to 100 ml graduated flask and make to volume. Allow mixture to stand 30 min. and filter thru a fluted filter. Pipet a 5 ml aliquot into a 200 ml volumetric flask and dilute to ca 175 ml with H<sub>2</sub>O. Add 2 ml of the Na hydrosulfite soln and 2 ml of the stannous chloride soln. Make up to volume, mix well, and allow to stand for 10 min. Pour soln into 1 liter Erlenmeyer flask and shake vigorously for 5 min. with access to air. Pipet 2 aliquots of 50 ml each into 100 ml Erlenmeyer flasks. Pour remainder into 100 ml flask but do not measure it.

#### Procedure B—Dried Skim Milk

*For materials containing casein.*—Proceed as directed in A except to use the acid-acetone soln for extraction instead of H<sub>2</sub>SO<sub>4</sub>. Also use a 50 ml aliquot for reduction with the Na hydrosulfite and stannous chloride.

#### DETERMINATION ON PFALTZ FLUOROMETER

Place portion of the standard quinine sulfate soln in cell of fluorometer and adjust iris diaphragm to give galvanometer deflection of 65.0, 50.0, 33.0, or 18.2, according to concentration of riboflavin expected in unknown soln. Place part of unknown soln in fluorometer, obtain galvanometer deflection, and calculate to riboflavin from calibration curve. This is (A). To one of 50 ml portions of unknown add with a pipet 1 ml of riboflavin soln (f) (2) and obtain the galvanometer deflection. Convert to riboflavin (B). To remaining 50 ml aliquot of unknown add 1 ml of the Na hydrosulfite soln, mix well, and read the deflection. Convert to riboflavin (C). To 50 ml of H<sub>2</sub>O add 1 ml of riboflavin soln (f) (2) and obtain deflection. Subtract deflection given by H<sub>2</sub>O from reading and convert to riboflavin (D).

#### CALCULATION

Use following formula to obtain p.p.m. of riboflavin:

$$\left( A - 1.02C \right) \left( \frac{1.02D}{1.02B - A} \right) F = \text{p.p.m. of riboflavin.}$$

A, B, C, and D are specified above, and F is dilution factor. No correction is necessary for the deflection caused by the reagents in the unknown. It is eliminated in the above calculation.

(4) In the tentative method for the determination of vitamin D for poultry (p. 372, 67), the following words in line 16 from end of paragraph were deleted: "all chicks that weigh 100 grams or less and,"

#### XXXVII. WATERS, BRINE AND SALT

The tentative method for the determination of fluorine (p. 529, 22) was revised as follows:

The first par. of 24 was changed to read:

Place 100 ml of sample in porcelain or Pt dish, make alkaline to phenolphthalein with the NaOH (avoid excess), and evaporate to 20 ml over a Bunsen burner just below boiling point.

The fifth sentence of the second paragraph was changed to read:

Connect flask with water condenser, add 20 ml of 60% HClO<sub>4</sub> to flask via the evaporating dish and funnel, and a quantity of a saturated soln of AgClO<sub>4</sub> that will

precipitate the chlorides (determined previously by titration with standard  $\text{AgNO}_3$  soln) and distil at  $132 \pm 3^\circ$ .

Also under par. 24, a third paragraph was added as follows:

Repeat the preparation and distillation, using 100 ml of distilled water in place of the sample, in order to ascertain the size of a blank.

Under par. 25 the first sentence was changed to read, "Prepare one standard, one color comparison tube, and one or more sample tubes as follows:

Par. 25(a) was changed to read 25(b) and in line 2, after the word "indicator," "1 ml of 0.1% hydroxylamine hydrochloride" was added.

A new paragraph was inserted as 25(a) to read as follows:

(a) *Color comparison tube*.—To 40 ml of  $\text{H}_2\text{O}$  add 2 ml of 0.05 N HCl, 1 ml of the alizarin red indicator, 1 ml of 0.1% hydroxylamine hydrochloride, and sufficient quantity of the  $\text{Th}(\text{NO}_3)_4$  to give faint but definite pink end point. Compare all end-point colors with this color.

The present 25(a) *Sample tube* then becomes 25(b).

Par. 25(b) was changed to 25(c), and in the second line after the word "indicator" the following words were added: "1 ml of the hydroxylamine hydrochloride."

### XXXVIII. RADIOACTIVITY

No additions, deletions, or other changes.

### XXXIX. DRUGS

(1) The following method was adopted as tentative for the separation and determination of aminopyrine, acetophenetidin, and caffeine:

#### AMINOPYRINE, ACETOPHENETIDIN, AND CAFFEINE

*Aminopyrine*.—Transfer 2.000 g of the powdered mixture into a separator, add 15 ml of 10% (W/V)  $\text{H}_2\text{SO}_4$  and 50 ml of  $\text{CHCl}_3$  and shake well. Draw off the  $\text{CHCl}_3$  into second separator and wash with 15 ml of 10%  $\text{H}_2\text{SO}_4$ . Filter the  $\text{CHCl}_3$  into a flask. Extract mixture in first separator with 5 more portions of 25 ml each of  $\text{CHCl}_3$ , washing each portion successively thru the diluted  $\text{H}_2\text{SO}_4$  as before and collect the  $\text{CHCl}_3$  in the flask. Test for complete extraction. Reserve this soln for determination of acetophenetidin and caffeine. Add acid washing in second separator to first separator. Render mixture alkaline with  $\text{NH}_3$  T.S., and remove the aminopyrine by successive extractions with 25 ml portions of  $\text{CHCl}_3$ . Wash each  $\text{CHCl}_3$  extract in a second separator with 5 ml of  $\text{H}_2\text{O}$  containing a few drops of the  $\text{NH}_3$  soln and filter solvent thru cotton into tared beaker. Evaporate solvent. Add a few ml of anhydrous ether to facilitate elimination of last traces of  $\text{CHCl}_3$  and again evaporate. Dry residue at  $80^\circ$  and weigh as aminopyrine.

*Acetophenetidin and Caffeine*.—Proceed as directed in 33(b), p. 570.

Examine residues obtained quantitatively to establish their identity.

(2) The following method for the determination of physostigmine salicylate was adopted as tentative:

**PHYSOSTIGMINE SALICYLATE****PREPARATION OF SAMPLE**

Count and weigh a representative number of tablets and calculate average weight. Grind to fine powder in mortar.

**DETERMINATION**

Weight accurately a sufficient quantity of the powdered material to contain ca 1 grain of physostigmine salicylate, transfer to a separatory funnel, and add enough  $H_2O$  (not exceeding 20 ml) to dissolve the material. Make alkaline to litmus with solid  $NaHCO_3$  and extract at once with  $CHCl_3$ , using 30, 20, 20, 10, and 10 ml portions. Transfer each extract to a second funnel containing 5 ml of  $H_2O$ . Wash each extract with this 5 ml of  $H_2O$  and filter into beaker, using, in stem of funnel, a cotton pledget moistened with  $CHCl_3$ . Test for complete extraction by making extra extraction with 5 ml portion of  $CHCl_3$  and treat separately as directed below. Evaporate combined  $CHCl_3$  extracts on a water bath, using current of air to assist evaporation. When volume has been reduced to ca 5 ml, remove beaker from bath and complete evaporation without aid of heat. Dissolve residue in a few ml of neutral alcohol. Add excess of 0.02  $N$   $H_2SO_4$ . Cover with watch-glass and heat on steam bath until the alkaloids have been washed down sides by the refluxing action. Remove watch-glass and evaporate bulk of alcohol. Cool. Add methyl red indicator and titrate excess acid with 0.02  $N$   $NaOH$ . Calculate equivalent physostigmine salicylate. 1 ml of 0.02  $N$   $H_2SO_4$  = 0.00826 g of physostigmine salicylate.

(3) The following microchemical tests for the detection of physostigmine, dilaudid, sulfapyridine, and sodium sulfapyridine were adopted as tentative:

**PHYSOSTIGMINE**

*Reagent. Gold bromide in HCl.*—To 1 g of gold chloride and 1.5 ml of 40%  $HBr$ , add  $HCl$  to make 20 ml. (Saturated soln of  $NaBr$  may be substituted for the  $HBr$ .)

*Preparation of sample.*—Add 1 mg of the substance to 1 drop of  $H_2O$  on a microscopical slide.

*Identification.*—Add a drop of reagent to side of drop containing the substance, apply cover-glass, and examine at a magnification of 100–150. The resulting crystals will consist of brown dendritic aggregates (fern-like).

**DILAUDID**

*Reagent. Na nitroprusside (solid).*

*Preparation of sample.*—Dissolve a minute quantity of the substance (less than 1 mg) in 2 drops of  $H_2O$ .

*Identification.* Into  $H_2O$  soln of the substance, drop minute fragment of  $Na$  nitroprusside. Crystalline precipitate immediately forms around the fragment, and upon microscopical examination at a magnification of 100–150, elongated 6-sided prisms will be observed, also occurring in aggregates.

**SODIUM SULFAPYRIDINE MONOHYDRATE**

*Reagent. Gold chloride soln.*—Dissolve 1 g of gold chloride in 20 ml of  $H_2O$ .

*Preparation of sample.*—Dissolve 1 mg of the substance in 2 drops of  $H_2O$  to make ca 1–100 soln.

*Identification.*—Place a drop of the reagent adjacent to water soln of the substance and draw it into margin of test-drop with a clean glass rod. Yellow rods, in x-shaped aggregates, will be observed upon microscopical examination at a magni-

fication of 100–150. After addition of reagent the drop becomes yellow, gradually darkening at the periphery. (Compare with sulfapyridine.)

#### SULFAPYRIDINE

*Reagent. Gold chloride soln.*—Dissolve 1 g of gold chloride in 20 ml of  $H_2O$ .

*Preparation of sample.*—To 1 mg of the substance on a microscopical slide, add 1 drop of acetone and 2 drops of  $H_2O$ , and stir material in with clean glass rod. (All material will not dissolve at once in this mixture, but there will be sufficient for microchemical test.)

*Identification.*—Place a drop of reagent next to test-drop of substance, drawing it gently with a clean glass rod, without stirring or covering, and examine microscopically at magnification of 100–150. Yellow rods or blades will be observed, also occurring in x-shaped aggregates. (The crystalline precipitate obtained with sulfapyridine is quite similar in habit to that characteristic of Na sulfapyridine with the same reagent, but the test-drop does not become brown at the periphery in the case of sulfapyridine. It is always observed, however, in testing for Na sulfapyridine monohydrate.)

(4) The following method for the determination of free iodine in iodine ointment was adopted as tentative:

#### FREE IODINE IN IODINE OINTMENT

Weigh to 1 mg ca 2 g of I ointment into a 250 ml I flask. Melt on water bath (not above  $70^\circ$ ), add 30 ml of  $CHCl_3$ , mix well, and then add 30 ml of  $H_2O$ . (All of base should be dissolved in the  $CHCl_3$  before  $H_2O$  is added.) Titrate with 0.1 N  $Na_2S_2O_8$ , using starch indicator, p. 44, 3(e). Approach end point dropwise, shaking flask vigorously to make sure that all iodine has been extracted from the  $CHCl_3$  layer. 1 ml of 0.1 N  $Na_2S_2O_8$  = 0.01269 g of I.

(5) The following method for the determination of potassium iodide in iodine ointment was adopted as tentative:

#### POTASSIUM IODIDE IN IODINE OINTMENT

Pour the liquids from the iodine determination into 500 ml iodine flask, rinsing flask with 200 ml of  $H_2O$ , divided into several portions. (It is desirable to maintain this volume within rather narrow limits.) Add 0.5 ml of 0.2% alcoholic *p*-ethoxy-chrysoidin indicator and 1–4 drops (to neutralize) of 0.1 N NaOH soln. (The aqueous layer should now be a clear yellow.) Titrate with 0.1 N  $AgNO_3$ , approaching end point dropwise and rotating flask frequently. (The  $AgNO_3$  soln causes turbidity due to the formation of colloidal AgI and development of a reddish-brown color similar to that observed in an overtitrated Volhard determination. The end point, which is produced by 1 drop of the volumetric soln, is characterized by flocculation of the colloidal AgI and complete disappearance of the reddish-brown tinge, which leaves an almost clear, pale yellow supernatant liquid.) Quantity (ml) of 0.1 N  $AgNO_3$  – ml of 0.1 N  $Na_2S_2O_8$  = ml consumed by the KI. 1 ml of 0.1 N  $AgNO_3$  = 0.0166 g of KI.

(6) The present tentative method for the determination of free iodine in iodine ointment (p. 619, 184, 185) was deleted.

### XL. MICROBIOLOGICAL METHODS

(1) The tentative methods for the examination of frozen egg products (p. 639) were revised as follows:



(a) The following paragraph was inserted under 1, "Sampling":

STERILIZATION OF GLASSWARE

Sterilize all glassware in oven sterilizer for a period of not less than 1 hour at temp. of 170–180°, or in autoclave steam pressure sterilizer for 20 min. at 15 lbs. pressure (121°).

(b) The parenthetical expression in line 1 under 2, "Procedure" was changed to read "(preferably square root of total)." Lines 15, 16, and 17 under the same paragraph were changed to read, "Refrigerate samples with dry ice (solid CO<sub>2</sub>) or other suitable refrigerant when analysis is to be delayed or sampling point is at some distance from the laboratory."

(c) The procedure for plate counts, 4, was modified to read as follows:

Inoculate one set of Petri plates with 1 ml portions of all dilutions from 1–10 to 1–1,000,000. Pour plates with nutrient agar, previously cooled to 40–45°. Incubate inoculated plates at 32° for 3 days. Count plates with the aid of a Quebec colony counter, if available. Express final results as numbers of viable microorganisms per gram of egg material.

(d) In 6, "Incidence of Hemolytic Staphylococci and Streptococci," the sentence, "Confirm the presence of coccus types of microorganisms by the microscopical examination of smears taken from representative hemolytic colonies and stained by Gram's method," was inserted directly after 37° in line 5.

(e) Section 8, "Tests for Fungi," was modified to read as follows:

Inoculate Petri plates with 1 ml portions of all serial dilutions from 1–10 to 1–100,000. Acidify melted Bacto-Malt Agar to pH 3.5 with 85% U.S.P. Lactic Acid as directed on the package label for Bacto-Malt Agar. Pour inoculated plates with acidified malt agar previously cooled to 40–45°. Incubate plates for 5 days at 20° or at room temp., if a 20° incubator is not available. Express final results as numbers of fungi per gram of egg material. Confirm yeast colonies by the microscopical examination of smears stained by Gram's method.

(f) Section 9, "Direct Microscopic Counts," was modified to read as follows:

*North's Anilin Oil Methylene Blue Stain.*

Soln 1—Anilin oil. . . . . 3.0 ml  
Soln 2—95% ethyl alcohol. . . . . 10.0 ml  
Mix Solns 1 and 2 and add 1.5 ml of HCl slowly with constant agitation. Add slowly 30 ml of a saturated alcoholic soln of methylene blue. Add H<sub>2</sub>O to give total volume of 55.5 ml. Filter.

Place 0.01 ml of the 1–10 or 1–100 dilutions of egg material on a clean, dry, microscopic slide, and spread over an area of 1 sq. cm. Permit the smear preparation to dry on a level surface at 35–40°. Immerse smears in xylene for 1 min. and dry in air. Immerse smears in 90% ethyl alcohol 1 min. and dry in air. Stain smears for 15 seconds with the North anilin oil methylene blue. Wash stained smears by repeated immersion in a beaker of H<sub>2</sub>O. Dry thoroly before examination. Observe subsequent procedure and precautions as directed in Standard Methods for the Examination of Dairy Products, 7th Ed., 1939, of the American Public Health Association. Multiply total count by 10 or 100, since the original smear preparation

was made from a 1-10 or 1-100 dilution. Express results as numbers of bacteria per gram of egg material.

(g) The directions for malt agar were changed to read as follows:

Malt extract, Difco, 3%, or..... 30.0 g  
Bacto-agar, 1.5%..... 15.0 g  
Distilled water..... 1000.0 ml  
Boil to dissolve medium.  
Sterilize at 15 lbs. pressure (121°) for 20 min. Final pH = 5.5.

NOTE: For detection of fungi in egg products malt agar should be acidified to pH 3.5 with 85% lactic acid U.S.P. after agar has been melted and prior to use. Medium should not be reheated after addition of acid.

(2) The following methods for the examination of canned fruits and other acid canned foods were adopted as tentative:

### EXAMINATION OF CANNED FRUITS<sup>1</sup> AND OTHER ACID CANNED FOODS

#### SAMPLING

The three primary objectives sought in the microbiological examination of canned foods that come within the pH range of "acid products" are the following:

- (1) Detection of spoilage.
- (2) Determination of commercial soundness (keeping quality).
- (3) Determination of sterility and the detection of type of bacteria capable of causing spoilage when the product is utilized as a sauce with other non-acid type foods.

The procedures for all three objectives, with respect to treatment of container, removal of sample, quantity of inoculum, and cultural methods are essentially the same. Modifications in treatment include the following:

(a) Normal appearing cans submitted for examination for commercial soundness or keeping quality should be incubated at 30° if less than 14 days has elapsed since the product was packed. Additional incubation at this temp. to insure at least 14 days' incubation is desirable. When no 30° incubator is available, incubation at an average room temp. of 25° may suffice.

(b) Samples examined for the presence of other than spoilage organisms require incubation at 37° in culture media suitable for the detection of organisms capable of causing spoilage of non-acid foods. Products such as tomato pastes, purées, and ketchups are often used as packing media (sauces) for non-acid products and may be responsible for spoilage under these changed conditions of pH.

(c) Samples submitted for examination for cause of spoilage should include direct microscopical examination as provided under 19(b), p. 644.

(d) Determination of pH is useful to supplement data for the detection of spoilage.

(a) *Physical examination and preparation of can.*—See 19(a), p. 644.

(b) *Removal of sample.*—(1) *Opening of container.*—See 19(b) (1).

(2) *Inoculum.*—Sample liquid or semi-liquid food products with sterile untapered pipets or inverted tapered pipets of suitable capacity. (The untapered pipets should have minimum dimensions of 350 mm in length and 5 mm inside diameter.) Sample solid or semi-solid food products with sterile spatulas, long-handled spoons, or other instruments,<sup>2</sup> depending on the character of the food under examination. Use pipets only for products of such viscosity as to permit transfer of the inoculum into the culture media by gravity. Use a sample consisting of a minimum of 15 grams or 15 ml of the food material and divide it into aliquots for duplicate culturing in each of the different culture media used in the examination.

<sup>1</sup> *This Journal*, 21, 454 (1938).

<sup>2</sup> *Ibid.*, 19, 431 (1936).

(3) *Sampling of product fractions.*—When representative inocula are desired from both the liquid and solid parts, transfer the solid component of the sample to the culture medium with forceps sterilized by flaming and use culture media in large test tubes (100×25 mm) or in wide-mouthed jars or bottles.

#### CULTURE MEDIA<sup>a</sup>

Because of their acid nature many products in this class are subjected to the limited processing designed only to prevent spoilage. This is done to preserve the color, flavor, and texture of the foods. In some instances the products are filled into the cans while hot and receive no further heat processing. Two main groups of micro-organisms encountered in the spoilage of this class of canned food products are the aciduric bacteria and the yeasts. Viable bacteria capable of producing spoilage in non-acid foods but rendered inactive in acid foods have frequently been responsible for the reporting of false positive results in examinations for spoilage in acid canned foods. Use the following media adjusted to a reaction below pH 5.0 for the detection of spoilage organisms:

##### 1. *Aciduric spoilage bacteria.*—

##### (a) *Buffered acid meat medium*

Distilled H <sub>2</sub> O . . . . .	1000 ml
	<i>grams</i>
Ground fresh lean beef . . . . .	500
Proteose peptone . . . . .	5
Sodium chloride . . . . .	5
Dextrose . . . . .	10
Potassium citrate . . . . .	12
Citric acid . . . . .	11

Infuse the beef-water mixture overnight in refrigerator. Heat in Arnold or boil for 30 min. Strain thru several layers of cheese cloth and press out broth, retaining meat press cake. Add distilled H<sub>2</sub>O to infusion to make up to 1 liter. Add the peptone and heat in Arnold or boil 10 min. Filter, and add salt. Acidify with potassium citrate and citric acid to pH 4.6, add the dextrose, and filter. Distribute the pressed-out beef remaining from infusion into medium sized test tubes (150×20 mm), ca 2 grams into each tube, and add 10 ml of the broth. Sterilize in autoclave at 15 lbs. pressure for 15 min. Prior to using, boil the tubed medium for 10 min. to expel adsorbed oxygen and cool promptly in a water bath.

The preparation of plate cultures is optional, and when desirable, the following plating medium is suitable:

##### (b) *Digest-yeast-tomato juice*

Tryptic milk digest . . . . .	100 ml
Bacto yeast extract . . . . .	2 g.
Tomato juice . . . . .	100 ml
Dextrose . . . . .	10 g
Agar . . . . .	15 g
Distilled water to make . . .	1000 ml

Prepare the tryptic milk digest as follows: To a liter of skim milk, add sufficient anhydrous Na<sub>2</sub>CO<sub>3</sub> to adjust the reaction to pH 8.0. Add a paste consisting of 2 g of Fairchild's trypsin, or 2 ml of freshly prepared enzyme. Add 25 ml of CHCl<sub>3</sub> and incubate at 30° for 24–48 hours. Agitate at intervals and maintain the optimum pH. After digestion neutralize with 10% HCl, and heat in a double boiler or on a water bath to remove the CHCl<sub>3</sub>. Sterilize 100 ml aliquots in autoclave for 15 min. at 15 lbs. pressure.

Dissolve by boiling, or heating, in an Arnold sterilizer, adding the tomato juice before sterilization. Sterilize in autoclave for 15 min. at 15 lbs. pressure.

<sup>a</sup> This Journal, 19, 440 (1936).

2. *Bacteria inhibited below pH 5.0.*—Use a medium essentially the same as the acid meat medium with reaction adjusted to pH 7.2, omitting the potassium citrate-citric acid mixture. (This medium, when rid of excess free oxygen by boiling and prompt cooling just prior to use, has been found satisfactory for the growth of anaerobes, aerobes, and facultative bacteria.)

3. *Yeast spoilage.*—

(a) *Clarified malt extract medium*

Dry malt extract (Difco) . . . 100 g  
Distilled water . . . . . 1000 ml

Dissolve the powdered malt extract in the H<sub>2</sub>O by heating in Arnold sterilizer, or on water bath. Adjust to pH 4.7 and cool to 50°. Add slowly 100 ml of a 5% suspension of Bentonite (colloidal clay) and mix vigorously. Hold at 50–75° for 30 min., then filter thru a fluted-paper filter until clear. Heat filtrate in autoclave 10 min. at 15 lbs. pressure, and filter through paper to remove any precipitate formed. Distribute into tubes, or flasks. For a plating medium, dissolve by heating 2% agar in the clarified broth, and filter if necessary thru cotton and cheese cloth. To avoid further precipitation sterilize at 10 lbs. pressure for 15 min. and cool promptly. (Bacto Malt Extract Broth can now be obtained in convenient dehydrated form and may be substituted for the above medium.)

4. *"Flat-sour" spoilage bacteria.*—See 14(a), p. 642.

INCUBATION

Incubate all cultures for the detection of spoilage organisms for at least 72 hours at 30°. For the detection of non-aciduric bacteria, incubate at 37° for at least 48 hours. For the occasional "flat-sour" spoilage encountered in such products as tomato juice, incubate for thermophilic anaerobes at 56° for at least 48 hours.

CULTURE STUDY

Use the Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists as a guide for study of microorganisms obtained in the cultural procedure described.

XLI. MICROCHEMICAL METHODS

The following microkjeldahl methods for the determination of nitrogen were adopted as tentative:

*Microkjeldahl Nitrogen Method*<sup>1</sup>

REAGENTS

(a) *Methyl red.*—0.1% ethanolic soln.

(b) *Sodium hydroxide and sodium thiosulfate soln.*—Aqueous soln of 40 g of NaOH and 5 g of crystalline thiosulfate per 100 ml.

(c) *Constant boiling hydriodic acid.*—N-free. Heat to 50° a mixture of 254 g of I and 185 ml of H<sub>2</sub>O in a 500 ml flask fitted with ground-joint condenser and dropping funnel. Add portionwise 60 g of 50% hypophosphorous acid at such a rate that the mixture gently boils. When I is reduced, apply heat and reflux the acid soln 3 hours while a liberal stream of CO<sub>2</sub> passes thru the soln. Change condenser to allow distillation and collect the constant boiling HI. Add 2 ml of 50% H<sub>3</sub>PO<sub>4</sub> and store in dark glass-stoppered bottles.

OPERATION OF DISTILLATION APPARATUS<sup>2</sup>

Generate steam in 1 (Fig. 2) by a resistance coil immersed in distilled H<sub>2</sub>O.

<sup>1</sup> *This Journal*, 16, 255 (1933).

<sup>2</sup> *Biochem. Z.*, 125, 253 (1921).

(The quantity of steam delivered is controlled, preferably, by a variable transformer or a sliding rheostat.

Close 3 with haemostatic forceps and 4 by removing the funnel from the wire hook and allowing it to hang so as to crimp the rubber connection. (Steam then passes thru trap 2 and distilling flask 5, and is condensed in 7 and collected in

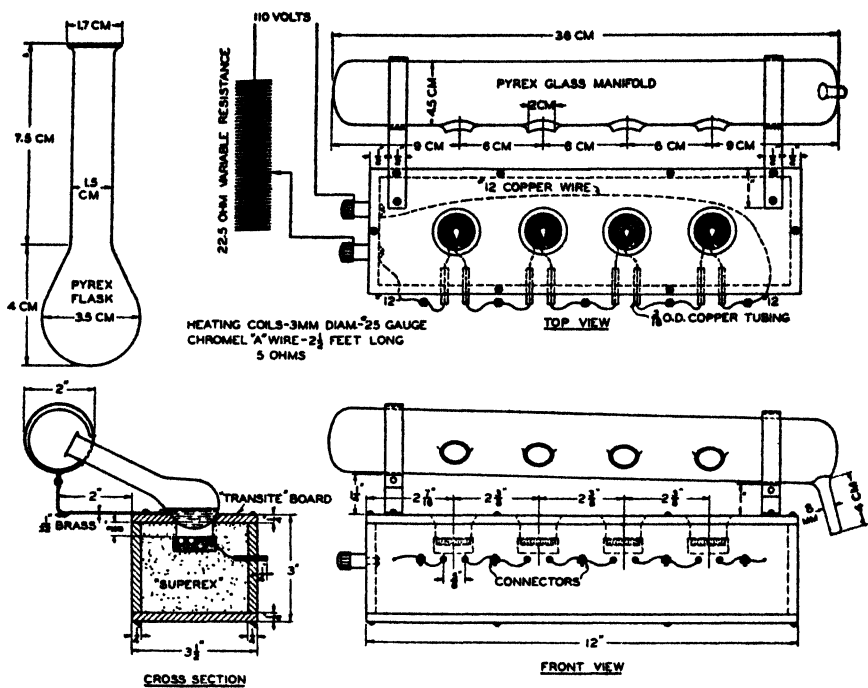


FIG. 1.—MICROKJELDAHL DIGESTER.

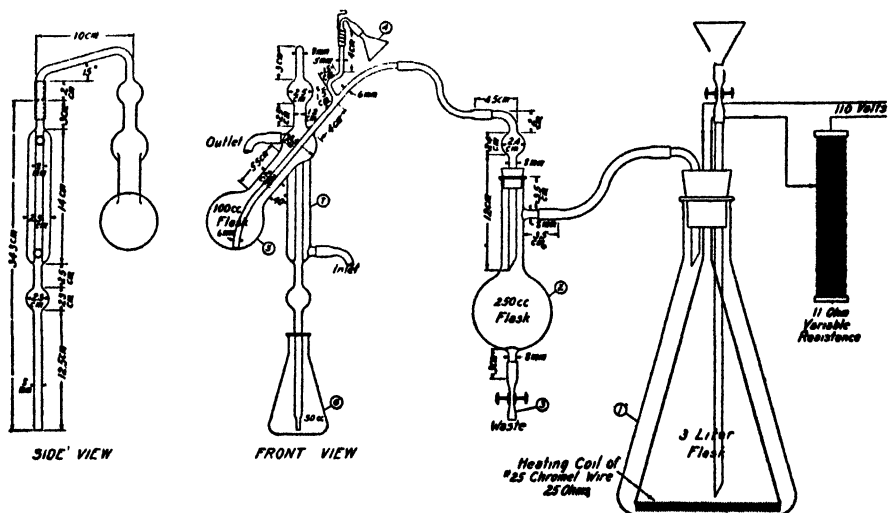


FIG. 2.—MODIFIED PARMAS-WAGNER MICROKJELDAHL DISTILLING APPARATUS.

6.) When the distillation is completed, remove spent liquor in 5 by breaking heating current. Immediately liquid in 5 is transferred to 2, remake heating current, open 3 to allow liquid in 2 to pass to waste and add  $H_2O$  to 5 thru 4. Close 3 and 4 again and repeat operation, which rinses 5, thus conditioning apparatus for next distillation.

#### DETERMINATION

Place in Kjeldahl flask, dimensions of which are shown in Fig. 1, ca 10 mg of substance, weighed upon a  $15 \times 25$  mm piece of cigaret paper; 40 mg of  $HgO$ , 0.5 g of  $K_2SO_4$ , and 1.5 ml of  $H_2SO_4$ . Gently heat flask and contents upon digester until frothing ceases, then increase temp. until acid mixture boils vigorously and vapors of acid rise to within 5 cm of mouth of flask. (Time from beginning to end of digestion should be 1 hour, and mixture should be colorless during the last 30 min. A longer combustion period does no harm.) Cool digest, add 1 drop of alcohol, and again heat mixture until it is colorless. (When acid mixture has cooled it is ready for distillation.)

Pass steam thru distilling apparatus for some time to assure proper working condition, then empty. Restart steam generator, open 3 and 4, and close rubber connection between 2 and 5 with haemostatic forceps. Dilute acid mixture in digestion flask with ca 8 ml of  $H_2O$ . Cover lip of digestion flask with a thin film of vaseline to prevent any liquid running down outside of flask, and transfer soln to 5 thru 4. Rinse flask with four 3 ml. portions of  $H_2O$  to make transfer quantitative. Next add sufficient of the  $NaOH$  thru 4 to neutralize acid and render final liquid strongly alkaline and wash it into distilling flask with 2 or 3 ml of  $H_2O$ .

Remove funnel from its support and allow to hang so as to crimp the rubber and thus close the system at this point. Close 3, open connection between 2 and 5, and place a small flame under 5. (Distillation begins almost immediately.) Collect condensate containing the  $NH_3$  in flask 6, containing 2 ml of 4% boric acid soln, to which is added 1 drop of the methyl red indicator.

Continue distillation with adapter under the acid soln until 8 ml of distillate is collected. Lower flask until adapter is above contents of flask, and in this position continue distillation until ca 1 ml more of distillate is collected. During this time wash outside of adapter with a little  $H_2O$  in a fine stream from a wash bottle. During preceding operations so adjust rate of distillation as to prevent boiling in 5 from mechanically carrying over any of its contents and temperature of liquid in receiving flask at end of the distillation from rising above  $40^\circ$ . Titrate the  $NH_3$  in the boric acid soln with 0.02  $N$   $HCl$ , using a buret graduated to 0.05 ml. Determine blank due to the reagents, and after subtracting this value from the buret reading calculate percentage of  $N$  in sample as follows:

$$\frac{(0.28) \text{ (ml of 0.02 } N \text{ HCl used)} (100)}{\text{Weight of sample}} = \%N.$$

#### *Friedrich method for N-N, NO, and NO<sub>2</sub> linkages\**

Weigh upon a piece of cigaret paper, as indicated in previous method, ca 10 mg of substance and place in digestion flask. Add 1 ml of the constant-boiling  $HI$  and gently reflux mixture during 45 min. Next apply more heat so that ca 0.7 of the  $HI$  slowly distils from the flask. Remove flask from digester and add 0.5 g of  $K_2SO_4$ , 1 ml of  $H_2O$ , and 1.5 ml of  $H_2SO_4$ . Heat mixture on digester until most of the  $H_2O$  is removed. Cool digest, add another ml of  $H_2O$ , and repeat distillation, the purpose being to remove the liberated  $I$  with steam; if this is not ac-

\* *Z. Physiol. Chem.*, 216, 68 (1933).

complished with 2 ml of  $H_2O$  add another ml and repeat process. Cool digest, add 40 mg of  $HgO$ , and complete digestion in usual way.

## XLII. STANDARD SOLUTIONS

(1) The method (official, first action) for the standardization of potassium permanganate (p. 653, 14, 15) was adopted as official (final action).

(2) The tentative specific gravity method for standardization of sulfuric acid solutions (p. 652, 13) was adopted as official (first action).

(3) The following method for the preparation of standard arsenous oxide solution was adopted as official (first action):

### ARSENOUS OXIDE SOLUTION

#### REAGENT

*Arseous oxide soln.*—Use National Bureau of Standards sample. Dry 1 hour at  $105^\circ$  immediately before using.

#### PREPARATION OF SOLUTION

Weigh the  $As_2O_3$  accurately by difference from small glass-stoppered weighing bottle (use ca 4.95 g per 1 liter for 0.1 *N* soln). Dissolve in normal  $NaOH$  soln (50 ml for each 5 g of  $As_2O_3$ ) in a flask or beaker by heating on steam bath. Add ca same quantity of normal  $H_2SO_4$ . Cool, and transfer mixture quantitatively to volumetric flask and make to volume. (Soln must be neutral to litmus, not alkaline.) Correct for volume changes due to temperature.

(4) The following method for the preparation and standardization of iodine solutions was adopted as official (first action).

### IODINE SOLUTION

#### PREPARATION OF STANDARD SOLUTION

Dissolve weighed quantity of I (12.7 g per liter for 0.1 *N* soln) and KI in the proportion of 20 g per 13 g of I in 50 ml of  $H_2O$ . When the I has dissolved, transfer soln to glass-stoppered graduated flask. Dilute to mark with  $H_2O$  and mix thoroughly. Keep soln in dark brown glass-stoppered bottle away from light and restandardize as frequently as necessary.

#### STANDARDIZATION

Transfer accurately measured portion of standard arsenous oxide soln (40–50 ml of ca 0.1 *N* soln for 0.1 *N* I soln) into an Erlenmeyer flask. Make slightly acid with  $H_2SO_4$  (1+10), neutralize with solid  $NaHCO_3$ , and add ca 2 g in excess. Titrate with the I soln, using ca 0.2% starch soln (5 ml per 100 ml) as indicator. Saturate the soln with  $CO_2$  at end of titration by adding 1 ml of the dilute  $H_2SO_4$  just before end point is reached.

From quantities of I and  $As_2O_3$  solns used calculate titer of I soln on basis of following relation:



(5) The following Mohr and Volhard methods for the standardization of silver nitrate were adopted as official (first action):

**SILVER NITRATE****PREPARATION OF SILVER NITRATE SOLUTION**

Dissolve slightly more than theoretical quantity of  $\text{AgNO}_3$  (equivalent weight, 169.89) in halogen-free  $\text{H}_2\text{O}$  and dilute to volume. Have all glassware thoroly clean, avoid contact with dust, and keep prepared soln in amber, glass-stoppered bottles and away from light.

*Mohr Method***REAGENTS**

(a) *Pure potassium chloride*.—Recrystallize  $\text{KCl}$  3 times with  $\text{H}_2\text{O}$ , dry at  $110^\circ$ , and then heat at ca  $500^\circ$  to constant weight.

(b) *Potassium chromate soln*.—5% soln of  $\text{KCrO}_4$  in  $\text{H}_2\text{O}$ .

**STANDARDIZATION**

Weigh accurately sufficient quantity of  $\text{KCl}$  to yield a titration of ca 40 ml (ca 0.3 g for 0.1 *N* soln) and transfer to 250 ml glass-stoppered Erlenmeyer flask with 40 ml of  $\text{H}_2\text{O}$ . Add 1 ml of the chromate indicator and titrate with the  $\text{AgNO}_3$  soln until the appearance of first perceptible pale red-brown color. Subtract from the titration the quantity of  $\text{AgNO}_3$  soln required to produce the end-point color in 75 ml of  $\text{H}_2\text{O}$  containing 1 ml of the chromate indicator. Calculate normality of the  $\text{AgNO}_3$  soln. Equivalent weight of  $\text{KCl}$  = 74.55.

*Volhard Method***REAGENTS**

(a) *Pure potassium chloride*.—See Mohr method.

(b) *Ferric alum soln*.—Saturated soln of  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ .

(c) *Nitric acid*.—(1 + 1). Dilute fresh  $\text{HNO}_3$  with equal volume of  $\text{H}_2\text{O}$ .

(d) *Nitric acid*.—2%. Prepare from fresh  $\text{HNO}_3$ .

(e) *Potassium or ammonium thiocyanate standard soln*.—Prepare ca 0.1 *N* soln from reagent that shows no chloride. Determine working titer by accurately measuring 40–50 ml of the standard  $\text{AgNO}_3$  soln, adding 2 ml of the ferric alum soln and 5 ml of the  $\text{HNO}_3$  (1 + 1) soln, and titrating with the thiocyanate soln until soln shows pale rose color after vigorous shaking.

**STANDARDIZATION**

Weigh accurately sufficient quantity of the  $\text{KCl}$  to yield a titration of ca 40 ml (ca 0.3 g for 0.1 *N* soln) and transfer to 250 ml glass-stoppered Erlenmeyer flask with 40 ml of  $\text{H}_2\text{O}$ . Add 5 ml of the  $\text{HNO}_3$  soln and run in excess of the  $\text{AgNO}_3$  soln. Mix, and allow to stand a few minutes protected from light. Filter thru Gooch crucible prepared with medium pad of asbestos previously rinsed with the dilute  $\text{HNO}_3$ . Wash the flask and precipitate with several small portions of the 2%  $\text{HNO}_3$ , passing washings through crucible until filtrate and washings measure ca 150 ml. Add 2 ml of the ferric indicator and titrate residual  $\text{AgNO}_3$  with the thiocyanate soln. From the titration, together with ratio of the two standard solns, calculate normality of the  $\text{AgNO}_3$  soln. (Errors of a blank are compensating and may be disregarded.) Equivalent weight of  $\text{KCl}$  = 74.55.

No report was given by the Committee on Standard Scale for Immersion Refractometer.



## REPORT OF THE COMMITTEE TO CONFER WITH AMERICAN PUBLIC HEALTH ASSOCIATION ON STANDARD METHODS OF MILK ANALYSIS

I reported last year that we had completed the 7th edition of Standard Methods for the Examination of Dairy Products. There has been no further collaborative work during the past year, but I have recently heard that the 8th edition of this publication is contemplated. Your committee will collaborate in the preparation of it, as usual, in so far as our official methods are concerned.

E. M. BAILEY, *Chairman*

Approved.

## REPORT OF A.O.A.C. REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE

For several years most of the projects conducted by the Crop Protection Institute have been aimed at discovering organic materials which would be efficient and satisfactory as insecticides or fungicides and harmless to man and animals. While organic materials have occupied the major place in the research program, they have not excluded important work with mineral substances.

Many of the problems involved a chemical study of the material being used, its reaction and physical effect on the crop and the character of the residue, if any, on the product.

The exploration studies have been a large, important and constructive field of endeavor. One company submitted about 600 organic compounds for tests. Of course, most of these could soon be discarded but several showed promising characteristics.

The development of apparatus and means for applying, spreading, and sticking the protective agency to the crop is often very important and involves both chemical knowledge and mechanical ingenuity.

The Crop Protection Institute conducted during the past year research projects for the following organizations:

1. *Carbide and Carbon Chemicals Corporation.*—
  - (a) Contact insecticides and fumigants.—Conducted in cooperation with the Indiana Experiment Station at Purdue University.
  - (b) Exploration studies of fungicides.—Conducted in cooperation with Boyce Thompson Institute.
2. *General Chemical Company, Inc.*—
  - (a) Field studies of Xanthone for codling moth and oriental fruit moth.—These were in cooperation with the Missouri, Iowa, and Arkansas Experiment Stations.
  - (b) Calcium arsenate with adhesive for cotton boll weevil.—Location, Georgia.

- (c) Copper compounds for strawberry leaf blight and for pepper blight.—Location, Louisiana.
- (d) Applications of sulfur and copper dusts with gypsum, with and without adhesives on peanuts and cantaloupes.—Location, North Carolina.
- (e) Study of a new form of nicotine.—Location, California, Florida, Indiana, Illinois, Massachusetts, Missouri, Oklahoma, Pennsylvania, and Texas.
- 3. *Roehm and Haas Company*.—  
Studies of red cuprous oxide and yellow cuprous oxide with and without adjuvants.—Location, Connecticut Experiment Station.
  - (a) Study of toxicity of dusts and sprays.
  - (b) Potato spraying with yellow cuprous oxide.
  - (c) Celery spraying and dusting.
  - (d) Tomato spraying and dusting.
  - (e) Cucumber spraying and dusting.
  - (f) Iris spraying.New apparatus has been devised.
- 4. *United States Rubber Company*.—
  - (a) Fungicide studies with organic compounds.—Location, Florida and Connecticut Experiment Stations.
  - (b) Insecticide studies with a new dermis spray.—Location, Florida, Connecticut, Massachusetts, Vermont, New Hampshire, New York, and Maine.
- 5. *Research Corporation of New York City*.—  
A chemical and biological essay of a group of plants that has shown toxic properties against insects.
- 6. *Miscellaneous*.—  
Some preliminary and exploratory work of a chemical nature has been done for several firms whose products seem to give some promise of usefulness for crop protection, but these have not developed far enough to be placed on a project basis.

Chemistry is constantly occupying an increasingly important place in the protection of crops from insects and diseases and the development of disease resistance in plants.

H. J. PATTERSON  
W. H. MACINTIRE

Approved.

## REPORT OF THE SECRETARY-TREASURER

By HENRY A. LEPPER

The year 1940 has seen the passing of a number of the members of our Association:

Thomas Stantial Gladding  
Frank Thomas Shutt  
Arthur W. Hanson  
Charles Chilton Moore  
John R. Eoff

The Committee on Necrology will report on these former colleagues.

Our attendance this year has not continued the record-breaking precedent established last year, but the registration of 525 shows the continued widespread interest in our work. This registration was achieved at a meeting devoted to prosaic business and without a special address as a program feature, which always attracts many additional guests.

Three prizes were granted to contestants for the Harvey W. Wiley Memorial Awards. The details were reported by a special committee. The Executive Committee directed the payment of the awards.

The Executive Committee approved an amendment to the Constitution, as follows:

Add to Article I, line 4, of Item 1 under Objects, between the words "products" and "caustic poisons," the word "cosmetics."

This amendment was adopted by vote of the Association.

A sum not to exceed \$500.00 was appropriated for the publication of a subject and author index for *The Journal* for volumes from 1930 to 1939, inclusive. The president was authorized to appoint a committee of three to consider clarification of the Constitution in regard to the eligibility and designation of referees. In the absence of G. E. Grattan, Chairman of Subcommittee A, H. H. Hanson was appointed for this year's meeting to complete the committee. The Editorial Board was authorized by the Executive Committee to establish a policy with respect to free subscriptions to *The Journal* for one year in response to requests from foreign countries unable to subscribe.

The report of the audit by a public accountant is submitted as the report of the treasurer.

#### STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED SEPTEMBER 30, 1940

BALANCE, OCTOBER 1, 1939		
Lincoln National Bank.....	\$ 2,759.70	
Montgomery Building and Loan Association.....	1,089.39	\$ 3,849.09
<hr/>		
RECEIPTS		
<i>Sales:</i>		
Methods of Analysis.....	\$ 2,339.30	
Journals.....	5,406.70	
Wiley's Principles.....	216.35	
Reprints.....	149.40	
	<hr/>	
	\$ 8,111.75	
Less: Discounts and allowances.....	827.53	
	<hr/>	
<i>Net sales</i> .....		7,284.22
<i>Other income:</i>		
Interest on investments.....	\$ 429.83	
Advertisements.....	346.50	776.33
	<hr/>	

*Miscellaneous receipts:*

Returned checks made good.....	\$ 305.40	
Books ordered through Association.....	1,596.72	1,902.12
		<u>\$13,811.76</u>

**DISBURSEMENTS***Expenses:*

Salaries.....	\$ 1,400.00
Postage.....	309.35
Association and meeting expense.....	382.65
Auditing.....	150.00
Exchange charges.....	10.90
Over and short.....	13.55
Printing and binding.....	5,018.16

*Total expenses* ..... \$ 7,284.61

*Miscellaneous disbursements:*

Books ordered through Association.....	\$ 1,702.73	
Returned checks.....	518.40	
Wiley awards.....	600.00	2,821.13

**BALANCE, SEPTEMBER 30, 1940**

Lincoln National Bank .....	\$ 2,593.05	
Montgomery Building and Loan Association.....	1,112.97	3,706.02
		<u><u>\$13,811.76</u></u>

**STATEMENT OF INCOME AND PROFIT AND LOSS  
FOR THE YEAR ENDED SEPTEMBER 30, 1940**

**INCOME***Sales:*

Advertisements.....	\$ 346.50
Journals.....	5,761.43
Methods of Analysis .....	5,008.25
Wiley's Principles.....	181.75
Reprints.....	149.40

*Total sales* ..... \$11,447.33  
 Less: Discounts and allowances..... 827.53

*Net sales* ..... \$10,619.80

*Cost of sales:*

Inventories, October 1, 1939.....	\$ 4,856.58
Printing and binding.....	11,525.55

\$16,382.13  
 Inventories, September 30, 1940.... 10,421.23 5,960.90

**GROSS PROFIT ON SALES** ..... \$ 4,658.90

**SELLING AND ADMINISTRATIVE EXPENSES:**

Salaries.....	\$ 1,400.00
Postage.....	309.35
Association and meeting expense.....	382.65
Auditing.....	150.00
Over and short.....	13.55
Bank exchange charges.....	10.90

<b>TOTAL EXPENSES</b> .....	<b>2,266.45</b>
<b>NET PROFIT ON SALES</b> .....	<b>\$ 2,392.45</b>
<b>OTHER INCOME:</b>	
Interest on investments.....	509.83
<b>NET PROFIT</b> .....	<b>\$ 2,902.28</b>

**REPORT OF AUDITING COMMITTEE**

The public accountant's audit of the books of the Association of Official Agricultural Chemists, Inc., for the year ending September 30, 1940, was examined by the Committee and found to be correct. Verification was also made of the bonds on deposit.

DAN DAHLE  
L. H. BAILEY

Approved.

**REPORT OF COMMITTEE ON WILEY MEMORIAL AWARDS**

The papers submitted by contestants for the Wiley Memorial Awards were appraised by each member of the committee charged with that responsibility. The conclusion of the committee is that the awards be given as follows:

*First:* "Determination of Potassium with Nitroso-R-Salt," Julius James Denzler, Purdue University, Lafayette, Ind.

*Second:* "A Comparison of the Hanus and the Rosenmund-Kuhnnehn Methods for the Determination of Iodine Numbers," Peter C. Duisberg, Pennsylvania State College, State College, Pa.

*Third:* "A Review of the Dithizone Method for Determining Mercury in Biological Materials," A. Zuckerman, McDonald College, Quebec, Canada.

The Committee has derived pleasure and benefit from its small contribution to the furtherance of intent to stimulate interest in chemistry in its relation to agriculture, and thereby to honor the memory of Harvey W. Wiley.

It is recommended that the awards of \$300, \$200, and \$100 go, respectively, to Messrs. Denzler, Duisberg, and Zuckerman.

W. H. MACINTIRE, *Chairman*  
E. M. BAILEY  
W. B. WHITE

Approved.

No report was given by the Committee to Cooperate with Other Committees on Food Definitions.

## REPORT OF COMMITTEE ON NECROLOGY

Since its previous meeting the Association has suffered the loss of five of its former members and associates.

The oldest of these was Thomas Stantial Gladding, who died December 8, 1939, in Easton, Maryland, after a short illness, at the age of 86. As a lifelong member of the well-known New York firm of Stilwell and Gladding, commercial fertilizer analysts, he took no part in the official proceedings of our Association. He was nevertheless greatly interested in its work and presented at our meetings papers of great importance on the analysis of fertilizers. Even before the Association of Official Agricultural Chemists was organized he was a contributor to the proceedings of the preceding convention of agricultural chemists at Atlanta in May, 1884, and to which meeting our present society is indebted for the impulse that led to its organization in Philadelphia on the following September 9th. At our Association's second meeting in September, 1885, Gladding presented two papers—one on "The Determination of Citrate-Soluble Phosphoric Acid in Natural Guanos," and the other on "The Estimation of Potash." These papers were of importance as they marked the beginning of the Association's work on the so-called Fresenius-Gladding method for citrate-insoluble phosphoric acid and on the better known Lindo-Gladding method for potash, both of which became recognized as official methods of fertilizer analysis. Papers on fertilizer analysis were also presented by Gladding at the 13th, 23rd, and 25th meetings of the Association. The last attendance of this venerable associate at our meetings was at the Golden Anniversary of the Association in October, 1934, when he was greeted with affection by many of his early colleagues.

Another lifelong member and loyal friend of our Association, who has recently passed away, is Dr. Frank Thomas Shutt, who died at his home in Rockcliffe Park, Ottawa, on January 5, 1940, in his eighty-first year. An obituary sketch of Dr. Shutt, who for many years ranked as Canada's most distinguished agricultural scientist, has been written by Mr. C. H. Robinson and is published in the current (November) issue of the *Journal* of the Association. Obituaries of both Gladding and Shutt were published in the *Analyst* for June, 1940.

Another recently deceased agricultural chemist, who was an almost constant attendant at our annual meetings during the twenty-year period between 1895 and 1915, is Charles Chilton Moore, Jr. He was a member of the staff of the old Bureau of Chemistry, having come to the Department of Agriculture at the suggestion of Assistant Secretary of Agriculture Dr. Charles W. Dabney, who was related to Moore by marriage. Moore was a collaborator on soils at the 1895 meeting of the Association, and he did

excellent work under Dr. Wiley for many years on the fertilizer requirements of American soils, the results of which, although written up, were never published owing to the opposition of another bureau. He was the author of articles in the *Journal of the American Chemical Society* on the determination of potash and on the study of available plant food. He also wrote two bulletins on cassava and sweet potatoes. Following his resignation in August, 1914, from the Bureau of Chemistry, Moore spent the last 25 years of his life as a consultant in food work in California. His sudden death in San Francisco on last February 29th at the age of 69 came as a shock to many friends.

The death of Arthur W. Hansen, Chief of the Seattle Station of the U. S. Food and Drug Administration from 1919 to 1931, and later Chief of the Los Angeles Station, should also be reported at this time. He was formerly an occasional attendant at our meetings. Illness obliged him to resign his position in November, 1934, after 27 years of service in Federal food and drug work. His health continued to fail and he finally passed away on July 29th of the present year.

John Ravenscroft Eoff, a former member of this Association, died at Niagara Falls, Canada, August 17, 1940. He was born at Ashland, Virginia, July 25, 1882, and was graduated from Virginia Polytechnic Institute, B.S., 1904. He entered service September 5, 1908, at Charlottesville, Virginia, and resigned January 10, 1913.

During his five years of service in the Department of Agriculture, Eoff was occupied with the study of wines and ciders. On these subjects he was co-author of a number of Bureau of Chemistry bulletins, dealing with the development of sugars and acids in apples and grapes during ripening. In these he furnished extensive analyses not only on acids and sugars, but also on ash constituents. Eoff discovered the presence of sucrose in American grapes. He participated in the work of the Association particularly on methods of analysis.

Eoff, because of his thorough knowledge of the manufacture and chemistry of wines, was at the time of his death vice-president and director of the Jordan Wine Company and the J. G. Bright Company of Canada.

C. A. BROWNE  
H. C. LYTHGOE

Approved.

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## REPORT OF NOMINATING COMMITTEE

The Committee on Nominations presents the following candidates:

*President:* L. B. Broughton, University of Maryland, College Park, Maryland.

*Vice-President:* J. W. Sale, U. S. Food and Drug Administration, Washington, D. C.

*Secretary-Treasurer:* W. W. Skinner, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

*Additional Members of the Executive Committee:* G. G. Frary, Vermillion, S. Dak.; J. O. Clarke, Chicago, Ill.; G. H. Marsh, Auburn, Ala.

H. R. KRAYBILL, *Chairman*

H. H. HANSON

C. C. McDONNELL

A unanimous vote was cast for the officers nominated.

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## REPORT OF COMMITTEE ON RESOLUTIONS

*Whereas* the 56th Annual Convention of the Association of Official Agricultural Chemists has now been completed; and

*Whereas*, an unusually large attendance has enjoyed and profited by the extensive interesting program;

*Be it resolved*, that the Association express its appreciation to Dr. W. W. Skinner for his splendid presidential address and the excellent manner in which he has conducted the convention; to Mr. H. A. Lepper for the various very valuable services rendered in so ably carrying on the work of the Secretary-Treasurer; to Miss Marian E. Lapp, and all officers of the Association for their valuable assistance in carrying on the work of the organization; to all section chairmen for their courteous and able management of their sections; to all Committees, Referees, Associate Referees, and their collaborators, in furnishing such a wealth of valuable and helpful reports; and to the Federal and State units for their interest in sending their representatives to this convention.

*Be it further resolved*, that our Secretary extend the thanks of the Association to the Managers of the Raleigh Hotel for the use of their rooms and for other acts of cooperation.

L. S. WALKER

ARTHUR E. PAUL

Approved.

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## MOLD MYCELIA COUNT OF BUTTER

In the publication of Table 3 of the paper on this subject by Vandaveer and Wildman, *This Journal*, 23, 698 (1940), the following footnote was omitted:

The total percentage of organoleptic grades of the creams under Columns 0, I, II, and III does not total exactly one hundred in samples under Churn Nos. 80, 72, 82, 88, 68, 74, 81, 83, and 86 because of the accumulative effect of rounding off the decimals and under Nos. 66, 89, and 87 because of the inclusion of rinsings added to the churns.—THE EDITOR.

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The following references were omitted when the paper entitled, "Absence of Reversion in Ammoniated and Limed Superphosphates of Low Fluorine Content", by MacIntire and Hardin, was published in *This Journal*, 23, 388 (1940):

## REFERENCES CITED

- (1) *Methods of Analysis*, A.O.A.C., 1935, 10.
- (2) BEESON, K. C., and ROSS, W. H., *Ind. Eng. Chem.*, 26, 992 (1934).
- (3) HARDESTY, J. O., and ROSS, W. H., *Ibid.*, 29, 1283 (1937).
- (4) JACOB, K. D., and ROSS, W. H., *J. Am. Soc. Agron.*, 23, 771 (1931).
- (5) JACOB, K. D., HILL, W. L., ROSS, W. H., and RADER, L. F., JR., *Ind. Eng. Chem.*, 22, 1385 (1930).
- (6) KEENEN, F. G., *Ibid.*, 1378.
- (7) KEENEN, F. G., and MORGAN, M. F., *Ibid.*, 29, 197 (1937).
- (8) MACINTIRE, W. H., *This Journal*, 16, 589 (1933).
- (9) MACINTIRE, W. H., U. S. Patent No. 2,067,538 (1937).
- (10) MACINTIRE, W. H., and SHUEY, G. A., *Ind. Eng. Chem.*, 24, 932 (1932).
- (11) MACINTIRE, W. H., and SHAW, W. M., *Ibid.*, 1401.
- (12) MACINTIRE, W. H., and SHAW, W. M., *J. Am. Soc. Agron.*, 26, 658 (1934).
- (13) MACINTIRE, W. H., and HAMMOND, J. W., *This Journal*, 22, 231 (1939).
- (14) MACINTIRE, W. H., and HARDIN, L. J., *Ind. Eng. Chem.* (In press).
- (15) MACINTIRE, W. H., HARDIN, L. J., and OLDHAM, F. D., *Ibid.*, 28, 48 (1936).
- (16) MACINTIRE, W. H., HARDIN, L. J., and OLDHAM, F. D., *Ibid.*, 711.
- (17) MACINTIRE, W. H., HARDIN, L. J., OLDHAM, F. D., and HAMMOND, J. W., *Ibid.*, 29, 758 (1937).
- (18) RADER, L. F., JR., and ROSS, W. H., *This Journal*, 22, 400 (1939).
- (19) ROSS, W. H., JACOB, K. D., and BEESON, K. C., *Ibid.*, 15, 265 (1932).
- (20) WILLARD, H. H., and WINTER, O. B., *Ind. Eng. Chem., Anal. Ed.*, 5, 7 (1933).

## ERRATA AND EMENDATIONS, METHODS OF ANALYSIS, A.O.A.C., 1940

The errors that are reported from time to time by those using this book will be published in *This Journal*. The following changes should be noted:

## Page Section

- 34, 47..... Change "44" to "48."  
 64, 109, line 14..... Transpose the sentence "Test filtrate," etc. to end of sentence following.  
 270, 10, line 1..... Change "1" to "2."  
 271, 15, line 2..... Change "precipiated" to "precipitated."  
 332, 69..... Add to title "Official, first action" and also the parenthetical statement "(Applicable to beverage concentrates)."  
 349, Notes, 4th par., 4th line from end.. Change " $\text{Na}_2\text{SO}_4$ " to " $\text{Na}_2\text{SO}_3$ ."  
 356, 22, line 3..... Change "2, 6, or 7" to "2 or 6."  
 357, 23, line 1..... change "2, 6, or 7" to "2 or 6."  
 371, 64, Col. 2, 4th line from bottom... Change "4.6" to "3.6."  
 386, 54..... Change 15 to 16.  
 416, 46(h)..... Delete this reagent  
 585, 78, 3rd line from bottom..... Delete "on steam bath."  
 637, Ref. 102..... Delete first 3 references and transpose first 2 to Ref. 104.  
 640, 7, line 1..... Change "media" to "medium."  
 722, Col. 8, next to last line..... Change 0.05676 to 1.05676.

The report on Changes in Methods (p. 66) should also be consulted.

## CONTRIBUTED PAPERS

### RAPID DETERMINATION OF SILICA IN ACID-INSOLUBLE SILICATES

By GEORGE McCLELLAN (Cosmetic Division, U. S. Food  
and Drug Administration, Baltimore, Md.)

In connection with work on the analysis of cosmetic powders, which normally contain talc or kaolin, a rapid method for the determination of silica in acid-insoluble silicates was formulated. It permits the use of strong acid for dissolving the fusion melt and dispenses with evaporation for dehydrating the silica. The single dehydration specified in the method yields a recovery of about 99.6 per cent.

The details of the method follow:

Weigh about 0.5 gram of the sample into a platinum crucible containing 6 grams of anhydrous  $\text{Na}_2\text{CO}_3$ , mix intimately, and place in a muffle furnace. Increase the temperature gradually until the contents of the crucible are in fusion, and continue to heat at  $950^\circ$ – $1050^\circ$  C. for 15 minutes.

Remove the crucible and allow it to cool. Dislodge the melt into a dry 400 ml. beaker. In a graduate mix 15 ml. of  $\text{HNO}_3$  with 5 ml. of water, and wash the crucible with small successive portions of this mixture, adding the washings to the beaker. (The melt dissolves rapidly, with the precipitation of most (about 95%) of the silica in the sample.)

Place the beaker under a hood and add, in the order named, 5 grams of  $\text{NH}_4\text{Cl}$ , 20 ml. of  $\text{HClO}_4$ , and 10 ml. of  $\text{H}_2\text{SO}_4$ . (When the  $\text{H}_2\text{SO}_4$  is added the mixture bubbles vigorously, and oxides of nitrogen are evolved.) Cover the beaker with a watch-glass, and boil over a moderate flame for 15 minutes after the evolution of oxides of nitrogen has ceased.

Cool the mixture, dilute with 150 ml. of hot water, and filter on an ashless paper. Wash paper and residue thoroughly with hot water, ignite the precipitate, and weigh as crude silica. Correct for impurities with HF in the usual manner.

### DISCUSSION

The melt is dissolved in nitric acid because of its solubility even in highly concentrated (12 *N*) solutions of this acid. It is obvious that the stronger the acid used, the more silica will immediately be precipitated and the less water there will be to remove during the subsequent dehydration.

Nitric acid is an inferior dehydrating agent. Hence, when it has fulfilled its purpose as a solvent it is displaced by the less volatile sulfuric and perchloric acids.

A combination of perchloric acid and sulfuric acid is used for dehydration because it gives a reasonably pure precipitate that filters rapidly. Perchloric acid alone gives a slow-filtering, almost pure precipitate, while sulfuric acid alone gives an impure granular precipitate that filters rapidly.

The ammonium chloride has a dual and possibly a triple purpose: it ensures smooth boiling, it helps to destroy the excess of nitric acid, and it may render the precipitation of silica more complete.

### RESULTS

The method outlined was used to determine silica in two samples of clay previously analyzed by the National Bureau of Standards. Sample No. 97, flint clay, was run in quadruplicate, and sample No. 98, plastic clay, was run in triplicate.

The results follow:

SAMPLE NO.	SiO <sub>2</sub> (AVERAGE RESULT OF NATIONAL BUREAU OF STANDARDS)	SiO <sub>2</sub> (BY METHOD OUTLINED)	RECOVERY (ON BASIS OF AVERAGE RESULT OF NATIONAL BUREAU OF STANDARDS)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
97	42.87	42.68	99.56
		42.70	99.60
		42.87	100.00
		42.71	99.63
98	59.11	58.90	99.64
		59.09	99.97
		58.87	99.59

The filtrates from the last two determinations of sample No. 97 were evaporated to fumes of perchloric acid, diluted, and filtered. The residues were ignited, weighed as crude silica, and corrected for impurities with hydrofluoric acid in the usual manner. The results follow:

SiO <sub>2</sub> PRESENT IN SAMPLE	RECOVERY BY FIRST DEHYDRATION		RECOVERY BY SECOND DEHYDRATION		TOTAL RECOVERY
<i>gram</i>	<i>gram</i>	<i>per cent</i>	<i>gram</i>	<i>per cent</i>	<i>per cent</i>
0.2178	0.2178	100.00	0.0010	0.46	100.46
0.2178	0.2170	99.63	0.0006	0.28	99.91

Two blanks were run to determine whether some of the silica recovered might come from reagents or equipment.

In blank No. 1, 6 grams of sodium carbonate was fused in a platinum crucible, dislodged into a 400 ml. beaker, and dissolved in 20 ml. of nitric acid (3+1). To the solution were added 5 grams of ammonium chloride, 20 ml. of perchloric acid, and 10 ml. of sulfuric acid. The mixture was boiled until oxides of nitrogen ceased to come off and for 15 minutes thereafter. When cool, the mixture was diluted with 150 ml. of hot water and filtered. Silica was determined in the residue.

Blank No. 2 was run in the same manner as No. 1, except that 40 ml. of perchloric acid and 20 ml. of sulfuric acid were used, and boiling was continued for 30 minutes after oxides of nitrogen had ceased to come off.

The results follow:

SiO<sub>2</sub> in No. 1 blank: 0.0001 gram.

SiO<sub>2</sub> in No. 2 blank: 0.0003 gram.

These figures indicate that recoveries by the proposed rapid method are actual and not the results of compensating errors.

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### A SURVEY OF METHODS FOR THE QUANTITATIVE ESTIMATION OF STARCH\*

By M. P. ETHEREDGE (Mississippi State Chemical Laboratory, State College, Miss.)

In order to make some direct starch determinations in this Laboratory it was thought that it might be well to make a survey of the literature, select the most suitable methods, and make determinations on commercial starches and also on some natural materials.

In 1898 Harvey W. Wiley and W. H. Krug (1) made a very complete report on starch methods. They were particularly interested in polarimetric methods so they tried the Guichard method, using nitric acid; the pressure method; the Baudry method; and a revision of the Reinke method, using hydrochloric acid. They believed that the Guichard method might be applicable to pure starches, but that the diastase method was the most promising. In general, they were opposed to polarimetric methods for either soluble starch or dextrose determinations. They also concluded that the sum of the several percentages obtained in the analysis of a cereal or cereal product does not approximate a hundred, and that variations are somewhat greater than they are in ordinary mineral analysis, but not so great as to warrant the assumption of the existence in cereals of a class of bodies of unknown properties and different in any marked degree from those known to exist.

Other attempts to bring starch into dispersive solution and get a quick quantitative estimation with the polarimeter include the use of hydrochloric acid alone in both concentrated and dilute solutions; hydrochloric with other acids, such as acetic; and sulfuric acid alone. According to Lintner (2) the sulfuric acid seems to give a different specific rotation with different starches.

For this investigation, it was thought best to use the current official methods. Two methods specifying hydrolysis by hydrochloric (3), and diastase-acid treatment (3), respectively, were taken from those of the Association of Official Agricultural Chemists. The Hopkins (4) revision of

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\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 28-30, 1940.

the old Mannich-Lenz (5) method was also used. A fourth method (unpublished, but obtained by private communication) was tried on some of the starches, but it was discarded when it failed to get all the starch.

#### EXPLANATION OF PROCEDURE AND OF SAMPLES USED

A saccharimeter (Bausch and Lomb make of the Lippich polarizer type) was used for all determinations, including the dextrose obtained from hydrolysis of the starch, although it was realized that the consensus of opinion is that the copper reduction method for determining dextrose is more accurate.

For the dextrose determination, one gram of material was used for each 100 ml. of solution. As the normal weight of sugar for the instrument is 26 grams (using a 200 mm. tube), the normal weight for dextrose would be  $66.5 \times 26 / 53.46$ , or 32.342 grams. As one gram of starch had been used for each 100 ml. of solution, and the factor to convert dextrose to starch is 0.9, then  $1^\circ \text{V.} \times 32.342 \times 0.9$  would produce the factor 29.1078 for converting to percentage of starch.

In the calcium chloride method, where the starch is dispersed, the factor 200 was used for specific rotation. This figure was proposed by Hopkins and was approximately the figure used by Wiley in 1898. The Committee on Starch of the Association of Official Agricultural Chemists also used this figure for their work on polarimetric methods. Therefore, the normal weight to be used here would be  $66.5 \times 26 / 200$ , or 8.645 grams for 100 ml. As 2 grams was used for each 100 ml. of solution, the Ventzke degrees would be multiplied by  $8.645 / 2$  or 4.3225 to obtain percentage of starch.

The pure starches used were the "Argo" brand (Corn Products Refining Company) and supposedly C. P. corn, wheat, and potato starch put out by Eimer and Amend. These starches were not prewashed. For a rough estimate of the purity of the starch samples, they were analyzed for moisture, ash, and protein, and the starch was obtained by difference. The moisture was determined by drying in a Freas oven for 24 hours at  $105^\circ$ – $106^\circ$  C. Experience in this laboratory has shown that this treatment compares favorably with drying in vacuo for a shorter period.

The natural materials used were cake flour, whole wheat flour, corn meal, whole rice, and lima beans. As these results were merely comparative, these materials were put through only an 80-mesh sieve instead of the 100-mesh sieve as recommended. When the calcium chloride method was used on the natural materials, instead of being centrifuged with alcohol, as recommended by Hopkins, they were washed on a filter paper, a platinum cone with a small amount of suction being used. Only two methods, the diastase-hydrochloric and calcium chloride dispersion, were used on these natural materials.

TABLE 1.—*Percentage of moisture, ash, and protein*

SAMPLE	MOISTURE	ASH	PROTEIN	STARCH (DIFF.)
Argo	10.06	0.09	0.31	89.54
C. P. Corn	8.24	0.09	0.35	91.32
C. P. Wheat I	10.21	0.21	0.29	89.29
C. P. Wheat II	5.32	0.18	0.29	94.21
C. P. Wheat III	9.77	0.19	0.29	89.75
C. P. Potato I	13.96	0.27	0.02	85.75
C. P. Potato II	15.33	0.27	0.02	84.38

TABLE 2.—*Percentage of starch by different methods*

SAMPLE	ACID HYDROLYSIS	DIASTASE-ACID METHOD	MANNICH-LENE METHOD (HOPKINS REV.)
Argo	80.34	80.34	89.26
	80.63	80.92	89.56
		81.21	
Mean	80.49	80.82	89.55
C. P. Corn	80.62	83.25	91.42
	82.37	84.70	91.55
			91.64
Mean	81.50	83.98	91.54
C. P. Wheat I	82.67	84.12	89.00
	84.41	84.41	89.17
		84.70	
Mean	83.54	84.31	89.09
C. P. Wheat II	80.05	80.05	92.98
	82.08	82.67	93.49
Mean	81.07	81.36	93.24
C. P. Wheat III	80.63	78.01	88.48
	80.92	80.92	88.52
Mean	80.78	79.47	88.50
C. P. Potato I	81.21	78.88	85.67
	82.67	81.21	85.89
			86.07
Mean	81.94	80.05	85.86
C. P. Potato II	79.76		83.90
			84.07
Mean			83.99

## RESULTS

Perhaps the varying moisture content of the different starches was due to length of exposure to the atmosphere and the humidity in the laboratory. Each starch was poured into a dish and exposed for several hours before being rebottled and determinations made therefrom. When C. P. Wheat II, which shows such a low moisture, was later exposed for a much longer period it took up about the same quantity of water as did the other samples. However, the original work was done when it showed the lower moisture content.

Wheat Starch Sample I bore a different stock number from Samples II and III, which were supposedly the same (out of different bottles), but Potato Starch Samples I and II, from different bottles, had the same stock number. With the exception of Wheat Starch Samples II and III, which were really the same, the results by the calcium chloride method check the starch by the difference shown in Table 1. Such comparative means are shown in Table 3.

## DISCUSSION OF RESULTS

Contrary to general statements in the literature, all the starch was not obtained in supposedly pure and commercial starches by either straight acid hydrolysis or by diastase treatment with subsequent acid hydrolysis. The checks were also very poor. In discussions with other analysts the writer has learned that they have experienced this difficulty. W. A. Noyes *et al.* (6) claimed to be able to find only 96–97 per cent of the total starch, and therefore proposed an arbitrary factor of 0.93 instead of the usual 0.9. Lintner and Düll (7) proposed the factor .94 to convert dextrose to starch. The results in this investigation are even lower than those cited. Perhaps part of the difficulty was owing to the use of the polarimeter instead of copper reduction. On the other hand, the diastase-hydrochloric method gave fairly good checks with the natural materials, and they more nearly agreed with results obtained by the calcium chloride method.

The calcium chloride method was very satisfactory in most instances on the pure starches. No particular trouble with foaming was experienced. The filtration was reasonably fast, and the solution was absolutely clear. On the natural material, foaming was harder to control and filtration was difficult. The foaming could be controlled by carefully watching the heat and using a larger flask. Filtration might be hastened by filter aids. The Association of Official Agricultural Chemists tried out this method to some extent in 1936 (8), 1938 (9), and 1939 (10). However, fewer samples were used, and no comparison was made with the official methods, other than the Rask method. Even Hopkins (9) with his initial revision of the Mannich-Lenz method, tried it on wheat products only. The writer tried corn, rice, potato, and a bean product in addition to the wheat products.

TABLE 3.—*Means of starch by difference and by calcium chloride*

SAMPLE	BY DIFFERENCE	BY CALCIUM CHLORIDE
Argo	89.54	89.55
C. P. Corn	91.32	91.53
C. P. Wheat I	89.29	89.09
C. P. Wheat II	94.21	93.24
C. P. Wheat III	89.75	88.50
C. P. Potato I	85.75	85.86
C. P. Potato II	84.38	83.99

TABLE 4.—*Percentage of starch in natural materials*

SAMPLE	DIASTASE-HYDROCHLORIC METHOD	MANNICH-LENZ METHOD (HOPKINS REV.)
Cake Flour	72.48	75.90
	73.06	75.90
	73.06	
Mean	72.87	75.90
Whole Wheat Flour	65.78	67.04
	68.11	67.26
		67.56
Mean	66.95	67.29
Rice Bran	6.11	7.91
	7.57	8.52
Mean	6.84	8.22
Corn Meal	62.29	63.50
	62.58	64.02
Mean	62.44	63.76
Whole Rice	71.31	77.29
	72.19	77.33
Mean	71.75	77.31
Lima Beans	45.41	42.45
	45.70	42.67
Mean	45.56	42.54

## QUALITATIVE TRIAL OF OTHER DISPERSION AGENTS

Other chemicals, which might be divided into organic liquids, inorganic salts, and solid organic acids, were used in an effort to prevent the serious difficulty in filtering.



One group of the organic solutions used apparently had no effect on starch. It included cellosolve, dioxan, chloroform, acetoacetic acid, diethylene glycol, ethyl malonate, ethyl oxalate, methyl ethyl ketone, and linalool. Another division that might be made (those organic solutions which had, apparently, a small amount of solvent action) included diacetone, methyl lactate, ethyl lactate, ethyl formate, ethylene glycol diacetate, propylene glycol, nitrobenzene, ethyl acetylglucolate, *o*-cresyl acetate, *o*-dichlorobenzene, and benzyl alcohol. A third classification included the following organic solutions, which seemed almost to dissolve the starch: ethylene glycol, safrole, methyl and ethyl phthalates, ethyl salicylate, eugenol, and isosafrole. Among the numerous salts and acids that were tried were fumaric acid, magnesium, sodium, and calcium acetate, succinic acid, *d*-1 malic acid, sodium citrate, magnesium sulfate, monocalcium phosphate, Rochelle salt, orthophosphoric acid, metaphosphoric acid, sulfanilic acid, sodium *p*-toluenesulfonate, sodium pyrophosphate, alum, *p*-hydroxybenzoic, *p*-nitrobenzoic, sodium borate, 3 per cent hydrogen peroxide, potassium bromide, sodium bromate, *d*-1 mandelic, semicarbazide hydrochloride, *o*-hydroxyquinoline, glycine, bleaching powder, magnesium nitrate, calcium nitrate, sodium salicylate, urea, potassium acid phthalate, sulfosalicylic, and formic acid.

The calcium nitrate and sodium salicylate methods showed possibilities, but it is doubtful whether either is an improvement over the calcium chloride method. The sodium salicylate filtered with even greater difficulty than did the calcium chloride when of a concentration sufficient to cause complete dispersion.

The sulfosalicylic and formic acids seem promising enough to warrant some quantitative results, and both filter quite easily. Further work is contemplated on these acids in this Laboratory.

### CONCLUSIONS

(1) It is believed that of the large number of methods that have been tried and published for the determination of starch, only the acid hydrolysis, the diastase-hydrochloric, and the Hopkins modification of the Mannich-Lenz procedure should be considered.

(2) The acid hydrolysis method cannot be used on natural materials, and for pure or commercial starches the revised Mannich-Lenz method gives more consistent results.

(3) The diastase-hydrochloric acid method also is inferior to the calcium chloride method when used on pure or commercial starches. However, with natural materials, the former gives fairly good results and perhaps should be used when there is too much filtering difficulty with the calcium chloride.

(4) The Hopkins revision of the Mannich-Lenz method seems to be the most promising single method. For commercial starches it gives better

and much quicker results than does any hydrolysis procedure. It is believed that by careful control of heating and perhaps the use of a filter aid, it can be used to good advantage on natural materials.

(5) It seems to be unnecessary to centrifuge the sample as recommended by Hopkins, and the slight change of putting the sample on filter paper and washing with aid of slight suction, using a platinum cone, could be used advantageously.

(6) It is believed that some other agent, such as formic acid, which was tried in this investigation, might be better than calcium chloride, particularly with natural materials.

#### ACKNOWLEDGMENT

The writer wishes to express his appreciation to W. F. Hand for suggesting this problem and for his constant interest and advice.

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### A NEW METHOD FOR THE DETERMINATION OF CHOLESTEROL AND ITS APPLICATION TO THE ESTIMATION OF THE EGG CONTENT OF ALIMENTARY PASTES\*

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Since 1916 advisory definitions and standards of identity for alimentary pastes have been issued in the United States (1-10). The general term "alimentary pastes" was dropped in favor of the classification "macaroni and noodles" in 1933. The standard for egg noodles or noodles has always specified a minimum percentage of egg solids. The present advisory standard (10) requires not less than 5.5 per cent of egg solids (whole egg and/or egg yolk) on the moisture-free basis.

The problem of determining the egg content of alimentary pastes is one that has engaged the attention of food chemists for many years. Stro-

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hecker and Vaubel (11) published an excellent review of the problem as a whole and of the work of the European investigators. Tillmans, Riffart, and Kühn (12) also discussed that work. Buchanan (13), in the only comprehensive report in English on the analysis of alimentary pastes of known composition, briefly reviewed the field, including work done in this country. It is therefore unnecessary to do more than state the deficiencies of the only method that has gained any significant attention in the United States, namely, that based on the determination of the lipoid (lipid) phosphorus. Even the average value for the lipoid phosphorus in flour (0.055 per cent on the dry basis) reported by Hertwig (14) constitutes a necessary correction that approaches in magnitude the amount of lipoid phosphorus contributed by the egg component in a noodle containing 5.5 per cent of whole egg solids on the dry basis. Moreover, the lipoid phosphorus content of alimentary pastes frequently decreases markedly on storage as reported in the above reviews. Hertwig (15) also observed a decrease in lipoid phosphorus in the course of the manufacturing process and suggested that the empirical factor 1.1 be used to correct the determined lipoid phosphorus value for such loss. Bornmann (16) and Mitchell (17) subsequently reported that the lipoid phosphorus in eggs may decrease very rapidly under certain conditions due to an enzymatic hydrolysis. In view of the findings cited, there is need for a method for the estimation of the egg content of alimentary pastes that will be more suitable for use in regulatory operations on such products. Tillmans, Riffart, and Kühn (12) had shown that the sterol determination is of value for this purpose and that the sterol content of alimentary pastes remains relatively constant during storage. Alfend (18), who has made valuable contributions to the development of analytical methods in this field, suggested that study of the sterol content of eggs, flour, and alimentary pastes be undertaken.

#### DETERMINATION OF CHOLESTEROL

For the present purpose a micro or semimicro cholesterol determination is required. A survey of the literature reveals a marked lack of agreement among analysts with regard to the reliability of the various micro methods. Abelin (19) and Wasitzky (20) published comprehensive reviews of these methods, and Ruiz and Torres (21, 22) made critical studies of a number of them. In general, the colorimetric methods require a close control of numerous factors, depend upon measurement of a transient color, and are particularly subject to interference by non-sterol substances. Lampert (23) noted the effects of interfering substances in some instances in applying such a method to the estimation of cholesterol in ice cream and similar difficulties prevented Tillmans, Riffart, and Kühn (12) from applying colorimetric methods to alimentary pastes. At the sacrifice of simplicity, the methods of Schoenheimer and Sperry (24) and of Sobel, Drecker, and Natelson (25) eliminated such non-sterol interference. Rif-

fart and Keller (26) applied a colorimetric method, using a step photometer, to the determination of sterols in alimentary pastes. They emphasize the necessity for exact adherence to the established procedure.

Quantitative organic microanalytical procedures require of the analyst considerable experience and manipulative skill in addition to painstaking attention to the purity of reagents. Tillmans, Riffart, and Kühn (12) applied the Szent-Gyorgyi (27) cholesterol method to alimentary pastes with unsatisfactory results. Riffart and Keller (26) obtained better results by application of the Yasuda (28) modification. However, these micro methods using digitonin are subject to the difficulties discussed below with respect to this reagent. Grossfeld (29) commented on the lack of other than micro methods in connection with the present problem.

The available semimicro methods were found to be based on the precipitation of cholesterol as a molecular compound with digitonin, a procedure introduced by Windaus (30) in 1908. Digitonin forms similar compounds with practically all naturally occurring sterols. Unfortunately it is an extremely expensive reagent and one of variable purity. Several investigators (31-35) found that the weight of the precipitate depends upon the excess and concentration of digitonin and that the excess required varies not only with different sterols but with different samples of digitonin, which means that a correction curve must be established for each sample of digitonin used. Terrier (35, 36) obtained unsatisfactory results in applying the digitonin method to the present problem.

#### CHOLESTEROL DIBROMIDE METHOD

Although the bromination of cholesterol had been previously described (37-39), Windaus (40) in 1906 was the first to report that cholesterol is precipitated from ether solution almost quantitatively as the dibromide (5-6 dibromo-cholestanol) by addition of a solution of bromine in glacial acetic acid while phytosterol bromide is precipitated under similar conditions only upon addition of considerable water. Several workers (41-43) commented on the application of this procedure qualitatively but published no data. Windaus and Hauth (44) found that stigmasterol tetrabromide precipitates under the same conditions as does cholesterol dibromide. The Windaus procedure has been widely used for the detection of cholesterol and stigmasterol, for the preparation of cholesterol dibromide in a number of syntheses, and in the purification of cholesterol. There has been some controversy (45-47) with regard to the purity of the cholesterol dibromide precipitate obtained by the Windaus method. A number of investigators (47-50) noted the labile character of the bromine in cholesterol dibromide. Ralls (51) reported that acetic acid should not be present during halogenation of cholesterol in the iodine number determination.

These facts suggested that a semimicro method might be developed

based on the titration of the bromine in cholesterol dibromide, which would neither require unduly expensive, difficultly obtainable reagents or apparatus nor include an empirical factor requiring frequent re-determination.

*Precipitation of Cholesterol Dibromide.*—The procedure adopted for the bromination of cholesterol and precipitation of cholesterol dibromide is based on studies of the suitability of numerous solvents, halogenating agents, and precipitants. In these studies the micro crystalline precipitates were dried to constant weight by aspiration with dry air. A semi-micro determination of the bromine in the precipitates was developed, involving evaporation with alcoholic potassium hydroxide solution, slight acidification with acetic acid, and titration of the bromide ion with 0.01 *N* silver nitrate, with eosin as indicator, as recommended by Kolthoff and Furman (52).

The cholesterol (melting point 147°–148° C.) used in the initial work was obtained from the Pfanstiehl Chemical Company, Waukegan, Ill. Schoenheimer (48) reported that cholesterol frequently contained small quantities of saturated sterols and described a method of removing them and a procedure for their determination. Pfanstiehl cholesterol was “purified” by that method, but only one precipitation was made because of the very small yield obtained. By Schoenheimer’s method the original and the “purified” Pfanstiehl cholesterol contained, respectively, 2.3 per cent and 2.2 per cent of saturated sterols. However, Gardner and Gainsborough (53) showed that this method gives false indications of 2–3 per cent of saturated sterols. De Fazi and Pirrone (54) found that cholesterol samples of different origins might behave differently toward bromination. Accordingly, the writer isolated cholesterol from fresh egg yolk and compared it with the Pfanstiehl cholesterol by the present procedure. No significant differences were found between the egg cholesterol and the original or “purified” Pfanstiehl cholesterol.

TABLE 1.—*Effect of bromine concentration and of temperature on the precipitation (20 mg. samples of Pfanstiehl cholesterol)*

BROMINE CONTENT OF THE CCl <sub>4</sub> SOLUTION	TEMPERATURE	PRECIPITATE	
		WEIGHT	BROMINE CONTENT
gram per ml.	° C.	mg.	per cent
0.1	0	25.4	27.9
0.2	0	25.4	27.8
0.4	0	24.8	27.9
0.6	0	24.8	28.0
0.8	0	24.3	27.8
0.2	–12	26.0	28.2
0.2	0	25.3	28.4
0.2	+10	24.7	28.3

The data (Table 1) show that close control of the bromine concentration or of the temperature is not a critical factor in the precipitation.

The theoretical yield is 28.2 mg. of cholesterol dibromide ( $C_{27}H_{46}O Br_2$ ) containing 29.26 per cent of bromine. There is a small loss due to solubility. The composition of the precipitate will be discussed later. Application of the procedure to quantities of 10–50 mg. of cholesterol showed that the weight of precipitate obtained was in a linear relation to the weight of cholesterol used. The average deviation from the regression line was 0.3 mg. The bromine content of the precipitate was constant within the limits of error of the bromine determination.

*Iodometric Estimation of Cholesterol Based on the Cholesterol Dibromide-Sodium Iodide Reaction.*—The argentometric bromine method gave accurate results, but considerable experience was required with the titration. Pirrone (50) and De Fazi and Pirrone (54) noted that iodine was liberated by the reaction of alkali iodide and cholesterol dibromide in acetone. They found 28.04 per cent of bromine in a cholesterol dibromide (29.26 per cent bromine) by application of the reaction on the macro scale. In the titration with thiosulfate solution they used large quantities of starch solution to detect the end point. In the present work on the semimicro scale no starch was used, since in the presence of some organic solvents the starch-iodine color is a far less sensitive indicator than the yellow color of iodine itself (52). Under these conditions, cholesterol dibromide was indicated to contain 28.6 per cent of bromine.

Extensive studies of various factors showed that the titer was independent of the volume of acetone and of the iodide concentration, within broad limits; was not affected by exposure of the reaction mixture to diffused light; and reached its maximum in less than an hour even in the presence of 1.5 ml. of water, the maximum present in the solution under the conditions of the method.

The results obtained by application of the precipitation and titration procedure to cholesterol are shown in Table 2, with the deviations from the weights of cholesterol calculated by the linear relation—

$$\text{mg. of cholesterol} = 1.0 + 2.16 \times (\text{ml. of } 0.01 \text{ } N \text{ } Na_2S_2O_3)$$

as obtained by the method of least squares.

This iodometric procedure is shorter and more precise than the argentometric procedure. Moreover, since only sterol bromides with the added bromine in the nuclear ring system give this reaction with sodium iodide readily (55), the interference of sterols having a double bond in the side chain is minimized. The application of this procedure to the analysis of eggs, flours, and alimentary pastes is discussed later under the appropriate headings referring to the original method. The chief disadvantage is the frequent occurrence of slight color in the acetone solutions obtained. This interference was considerably reduced by a purification of the sterol extracts by adsorption on alumina as described later.

TABLE 2.—*Iodometric cholesterol determination based on the cholesterol dibromide-sodium iodide reaction*

DESCRIPTION OF SAMPLE	WEIGHT SAMPLE	TITER 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$	WEIGHT CALCULATED	DEVIATION
	mg.	ml.	mg.	mg.
Egg Cholesterol	5.0	1.82	4.9	+0.1
" "	10.0	4.10	9.9	+0.1
" "	15.0	6.60	15.3	-0.3
" "	20.0	8.95	20.3	-0.3
Pfanstiehl Cholesterol	5.0	1.82	4.9	+0.1
" "	5.0	1.85	5.0	0.0
" "	10.0	4.10	9.9	+0.1
" "	10.0	4.08	9.8	+0.2
" "	15.0	6.46	15.0	0.0
" "	15.0	6.57	15.2	-0.2
" "	20.0	8.86	20.1	-0.1
" "	20.0	8.76	19.9	+0.1
" "	40.0	17.92	39.7	+0.3
Average deviation				0.15

*Interference of Wheat Phytosterols.*—The very low sterol content of flour makes difficult the isolation of adequate quantities of sterols with which to work. The meager information available indicates that they are mixtures of sitosterols and dihydrositosterol (56–58). Wheat germ oil is a better source of a similar very complex mixture containing at least four well characterized unsaturated phytosterols and dihydrositosterol (59–64). The interference of such phytosterols should give a rigorous test of the cholesterol method with sterols representative of those that might be found in farinaceous products. Accordingly the sterol fraction was isolated from wheat-germ oil. Application of the cholesterol method showed that there was coprecipitation of cholesterol and phytosterol dibromides. The results of numerous experiments with phytosterol alone and admixed with cholesterol are plotted in the graph, Figure 1. The quantities of cholesterol used in the four series were 0, 5, 10, and 20 mg., respectively, beginning with the first series from the bottom. The weight of precipitate as cholesterol (ordinate) was determined by titration and calculated according to the relation—

$$\text{mg. precipitated (as cholesterol)} = 2.16 \times (\text{ml. of } 0.01 \text{ N } \text{Na}_2\text{S}_2\text{O}_3).$$

The deflections in the curves for mixtures containing the smaller quantities of cholesterol when the ratio of phytosterol to cholesterol is about 1.25:1, together with the apparent presence of only about 80 per cent of sitosterol in the phytosterol, indicate that these deflections occur when the ratio of cholesterol to sitosterol is 1:1. The results obtained could be satisfactorily interpreted on the basis of the formation of a molecular

compound between the dibromides of cholesterol and sitosterol of the type that Lettré (65) has shown to form between closely related sterols. Coprecipitation of the dibromides of cholesterol acetate and phytosterol acetate also occurred.

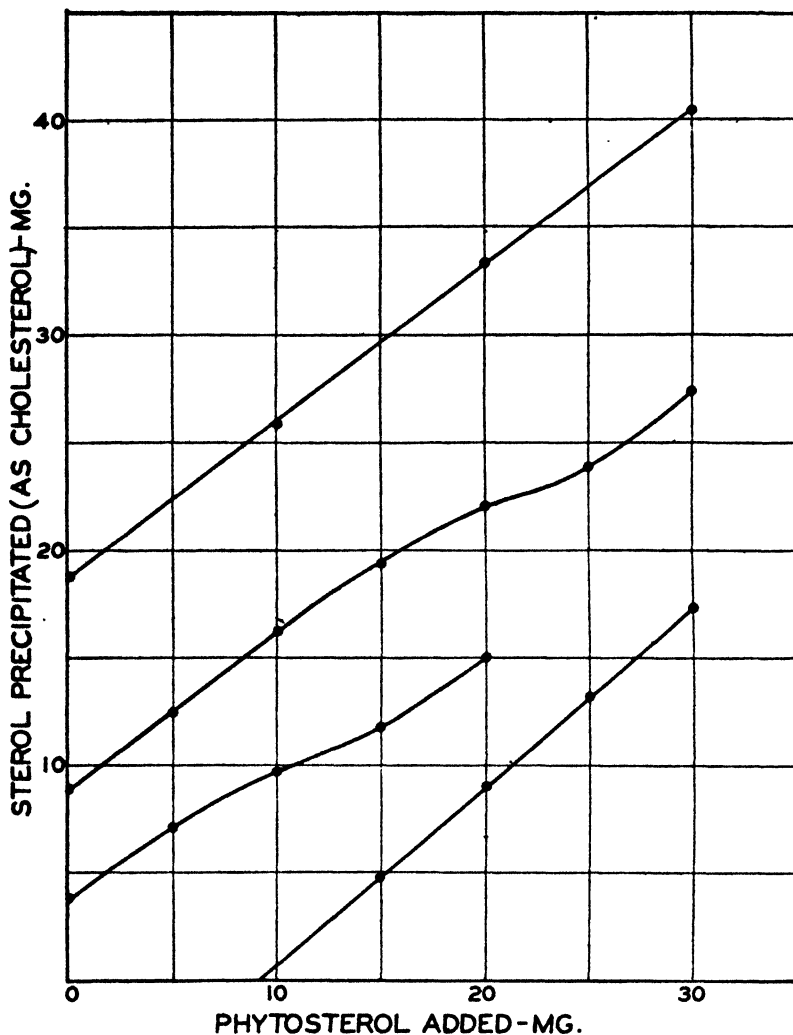


FIG. 1.—COPRECIPITATION OF CHOLESTEROL AND PHYTOSTEROL.

For the present work, the significant fact is that the amount of sitosterol dibromide that precipitates is independent of the quantity of cholesterol present so long as this equals or exceeds the sitosterol content of the sterol mixture. Hence in determining the sterol content of farinaceous products containing little or no egg, sufficient cholesterol must be added to the unsaponifiable matter to assure that an excess is present.



*Preparation of Pure Cholesterol Dibromide and Pure Cholesterol.*—Pure cholesterol dibromide is not isolated under the conditions of the present method (Table 1), though evidence will be presented later to show that it is quantitatively formed. Lifschütz (46) found that the precipitate obtained by the Windaus method held loosely bound acetic acid. The writer required pure cholesterol dibromide in the development of the oxidative bromine procedure discussed below, but was unsuccessful in obtaining it by numerous trials of methods described in the literature (40, 45, 46, 54). The labile character of the bromine causes the chief difficulty in obtaining pure cholesterol dibromide, and it seems that molecular compounds of the dibromide and its decomposition products may occur. The present work indicated that the preparation should be obtained pure without recrystallization and in a dry state if possible. Accordingly, a new method of preparation was developed (Experimental Part); it yields the pure dibromide as shown by its bromine content and its melting to a clear colorless liquid. Preparations obtained by other methods had decomposed before or on melting.

Cholesterol of unquestionable purity is obtainable from the pure dibromide by debromination under mild conditions. Schoenheimer (48), in purifying cholesterol, precipitated the dibromide (Windaus method), recrystallized it from alcohol, and debrominated it by boiling with sodium iodide in alcohol. He repeated the process with the regenerated cholesterol, obtaining yields of 10–15 per cent. The instability of cholesterol dibromide makes it inadvisable to boil it in alcohol during a purification process. The dibromide can be debrominated smoothly at room temperature by the action of sodium iodide in acetone. The yield of cholesterol purified through the dibromide by the new procedure was 63 per cent. It is emphasized that the Pfanstiehl cholesterol used probably was free of saturated sterols, and removal of these from cholesterol was the primary purpose of Schoenheimer's process. However, the debromination in either method is preferably carried out by the sodium iodide reaction in acetone at room temperature.

*Iodometric Estimation of Cholesterol Based on the Oxidative Bromine Determination.*—Subsequent to the application of the original method to the analysis of alimentary pastes, Kolthoff and Yutzy (66) published the rapid and accurate modification of the van der Meulen (67) method for determining micro quantities of bromides. Busbey and Drake (68) had applied a similar procedure to the determination of methyl bromide in air. The van der Meulen method yields for titration six equivalents of iodine for each equivalent of bromine. By adjustment of the reagents it was found that the Kolthoff-Yutzy procedure gave very satisfactory results for the determination of up to 40 mg. of bromine. The revised procedure was applied to pure cholesterol dibromide, with the results shown in Table 3.

TABLE 3.—*Analyses of cholesterol dibromide by the oxidative iodometric method*

CHOLESTEROL DIBROMIDE ( $C_{27}H_{48}O Br_2$ )	TITER 0.02 N $Na_2S_2O_3$ (CORRECTED FOR BLANK)	BROMINE
mg.	ml.	per cent
5.65	6.24	29.4
10.15	11.15	29.3
14.95	16.50	29.4
20.05	22.08	29.3
30.25	33.30	29.3
36.65	40.27	29.3
Average		29.3
Calculated for $C_{27}H_{48}O Br_2$		29.26

The revised procedure was applied in the cholesterol dibromide precipitation method to determine the relation between the purified cholesterol and the titer. The results are shown in Table 4 with their deviations from the values calculated by the relation—

$$\text{mg. of cholesterol} = 0.55 + 0.688 \times (\text{ml. of } 0.02 \text{ N } Na_2S_2O_3),$$

as obtained by the method of least squares.

TABLE 4.—*Iodometric cholesterol determination by the oxidative bromine method*

CHOLESTEROL USED	TITER 0.02 N $Na_2S_2O_3$ (CORRECTED FOR BLANK)	CHOLESTEROL CALCULATED	DEVIATION
mg.	ml.	mg.	mg.
5.40	7.24	5.55	-0.15
4.90	6.39	4.95	-0.05
5.20	6.92	5.30	-0.10
9.95	13.64	9.95	0.00
10.20	13.90	10.10	+0.10
9.75	13.33	9.70	+0.05
15.40	21.80	15.55	-0.15
14.80	20.46	14.60	+0.20
15.10	21.02	15.00	+0.10
20.40	28.93	20.45	-0.05
20.00	28.32	20.00	0.00
19.95	27.88	19.70	+0.25
29.95	43.01	30.10	-0.15
30.30	43.43	30.20	+0.10
30.45	43.32	30.35	+0.10
39.95	57.01	39.75	+0.20
39.80	57.10	39.80	0.00
40.15	57.63	40.15	0.00
50.10	72.20	50.20	-0.10
Average deviation			0.10

The theoretical factor in this determination for converting the 0.02 *N* sodium thiosulfate titer to cholesterol is 0.6444 instead of 0.688, as found in the equation from the experimental data, indicating that only 93.7 per cent of the theoretical amount of cholesterol dibromide was actually formed and precipitated. With cholesterol acetate only 94.4 per cent of the dibromide was precipitated, showing that oxidation of the secondary hydroxyl group does not account for the discrepancy. Moreover, with 10–40 mg. samples of cholesterol all the unprecipitated cholesterol dibromide was recovered from the filtrates by dilution with water. The theoretically expected amount of dibromide was also obtained by using 50 per cent (instead of the usual 80 per cent) acetic acid as the precipitant. These facts suggest the existence in the solution of an equilibrium between two forms of cholesterol dibromide, probably *cis* and *trans* isomers, one of which is less soluble than the other.

From the standpoint of the objectives of the present work, the iodometric method based on the oxidative procedure is believed to be the most suitable for the cholesterol determination. It is sufficiently precise and accurate, and the titration end point is familiar to all analysts and is not affected by the presence of colored matter in the precipitates. The revised oxidative method requires more time than does the sodium iodide method, and it may be affected to a greater extent by any non-sterol bromides that might precipitate.

#### ESTIMATION OF THE EGG CONTENT OF ALIMENTARY PASTES BY MEANS OF THE CHOLESTEROL DETERMINATION

##### *Cholesterol Content of Hen's Eggs*

The cholesterol of hen's eggs occurs practically entirely in the yolk. Dam (69) and Kusui (70) found only a trace in the entire white of an egg. Tillmans, Riffart, and Kühn (12) concluded from a limited number of analyses by a rather unsatisfactory method that all the cholesterol was present in the free form. However, studies by Mueller (71), Thannhauser and Schaber (72), Dam (73), and Kusui (70) indicate that at least 10 per cent of the sterol in fresh eggs may be esterified. Dam (69) found 0.468–0.610 per cent of total cholesterol in individual eggs. Tillmans, Riffart, and Kühn (12) reported an average of 1.49 per cent of free cholesterol in eight samples of egg yolk. Lampert (23) found an average of 1.36 per cent of total cholesterol in the yolk. Perlman (74) reported 0.54–0.72 per cent of free cholesterol in twelve individual eggs, the average being 0.60 per cent. Riffart and Keller (26) found the average free cholesterol in three samples of yolk to be 1.49 per cent (titrimetric method) and 1.50 per cent (colorimetric method).

(1) *By the Original Method.*—Dam (75) found that the total cholesterol in eggs could be extracted with ether after heating the egg with 60 per cent potassium hydroxide solution for 2–3 hours. This method was

adapted to the present work. The unsaponifiable matter is extracted by a procedure developed from the Kerr-Sorber method (76) as modified by Hertwig, Jamieson, Baughman, and Bailey (77), and the new cholesterol method is then applied to the unsaponifiable matter.

In Table 5 are shown the results obtained by the original method on eggs used in the preparation of alimentary pastes of known composition.

TABLE 5.—*Cholesterol content of eggs used in authentic alimentary pastes*

DESCRIPTION OF SAMPLE	ORIGINAL METHOD			ADSORPTION MODIFICATION			REVISED METHOD		
	TOTAL SOLIDS	UNSAPONI- FIABLE MATTER IN THE SOLIDS	CHOLES- TEROL IN THE SOLIDS	TOTAL SOLIDS	ADSORBED UNSAPONI- FIABLE MATTER IN THE SOLIDS	CHOLES- TEROL IN THE SOLIDS	TOTAL SOLIDS	UNSAPONI- FIABLE MATTER IN THE SOLIDS	CHOLES- TEROL IN THE SOLIDS
Frozen Whole Egg	<i>per cent</i> 27.39	<i>per cent</i> 3.10	<i>per cent</i> 2.23	<i>per cent</i> 27.49	<i>per cent</i> 2.38	<i>per cent</i> 2.26	<i>per cent</i> 26.87	<i>per cent</i> 2.86	<i>per cent</i> 2.20
		2.85	2.34		2.36	2.26		2.84	2.19
		(3.14)*	(2.19)					2.88	2.19
		(3.14)	(2.30)						
							26.73	2.78	2.25
								2.79	2.26
Frozen Yolk	46.54	3.87	2.97	46.67	3.12	2.98	46.15	3.43	2.97
		4.30	3.03		3.07	3.00		3.45	2.97
		3.93	3.01					3.50	2.96
							46.10	(3.56)	(2.94)
								(3.82)	(2.98)
								(3.59)	(2.97)
Chinese Dried Yolk	97.35	3.56	2.89	97.35	2.95	2.87	96.97	3.45	2.81
		3.68	2.85		2.97	2.86		3.39	2.80
							96.95	(3.62)	(2.84)
								(3.57)	(2.79)

\* All values in parentheses were obtained when the samples were subjected to acid hydrolysis before saponification.

The results by the original method (Table 5) were not quite so satisfactory as desired. In earlier work the writer had found that an efficient aluminum oxide adsorbent for cholesterol can be prepared by ignition of basic aluminum acetate powder. Accordingly, the unsaponifiable matter was purified by adsorption on alumina from petroleum benzine solution and elution of the adsorbed matter with ether. It was apparent that all

the adsorbed matter was not eluted by the ether, but the sterol was recovered free of most of the colored substances that interfered with the titration. This adsorption modification yielded more precise results (Table 5). After these analyses were made in the spring of 1936 the frozen eggs were stored at about  $-10^{\circ}$  C., and the dried yolk was stored at about  $5^{\circ}$  C. until the spring of 1940.

(2) *By the Revised Method.*—The oxidative iodometric procedure eliminated the interference noted in the cholesterol dibromide-sodium iodide procedure due to the color in acetone solutions of the precipitates. The adsorption modification, which had been developed to reduce this interference in the latter procedure, was omitted from the revised method. Further study of the method for extracting the unsaponifiable matter showed that its precision was greatly improved by filtration of the ether solutions of the unsaponifiable matter through anhydrous sodium sulfate. This removed finely divided calcium soaps that could not be completely filtered out by the cotton used in the original method.

The results obtained by the revised method in the spring of 1940 on the same eggs previously analyzed in 1936 are also shown in Table 5. After each series of analyses the thawed eggs were refrozen, so the variation in results between series includes those errors involved in the preparation of the samples for analysis. The method developed for use on alimentary pastes includes an acid hydrolysis before the saponification. The results in parentheses in Table 5 show that such acid hydrolysis does not significantly affect the cholesterol determination in eggs.

During 1936, samples of commercial frozen eggs that had been collected at egg-packing plants in various parts of the country by inspectors of the Food and Drug Administration were sent to Washington, D. C., in the frozen condition. These samples were stored at about  $-10^{\circ}$  C. because analyses could not be made at the time. Since the results obtained on the eggs used in the preparation of the authentic alimentary pastes showed no significant differences under like storage conditions for the same period, a number of the commercial samples were analyzed by the revised method. In addition, there were analyzed a few samples collected from storage lots of the 1939 pack of three different packers and also several samples of dried egg yolk. The fat by acid hydrolysis (78) was also determined. The results of these analyses are given in Tables 6, 7, and 8. The cholesterol content varies to about the same extent as the content of other egg yolk constituents in commercially broken out eggs as reported by Mitchell, Alfend, and McNall (79). Moreover, all the cholesterol values for the 1939 pack come within the limits found for the 1936 pack.

The results obtained demonstrate (1) that the cholesterol content of commercially broken out whole eggs and yolks does not vary enough to preclude the use of this factor as an index of the egg content of foods; (2) that the cholesterol content of frozen eggs and dried eggs shows no

TABLE 6.—*Cholesterol content of commercial frozen whole eggs*

SAMPLE NO.	YEAR PACKED	SOURCE OF SHELL EGGS (STATE)	TOTAL SOLIDS	FAT BY ACID HYDROLYSIS IN SOLIDS	UNSAPONIFI- ABLE MATTER IN SOLIDS	CHOLESTEROL IN SOLIDS	CHOLESTEROL IN FAT
1	1936	Neb.-Iowa	per cent 26.84	per cent 45.12	per cent 2.75	per cent 2.13	per cent 4.72
2	"	?	27.26	45.67	3.17	2.23	4.89
3	"	California	27.73	48.21	2.68	2.13	4.42
4	"	Mo.-Kan.	25.81 (25.65)*	43.63 —	2.60 (2.61)	2.08 (2.06)	4.77 —
5	"	Mo.-Kan.	26.85	45.51	2.77	2.13	4.69
6	"	California	26.50 (26.65)	46.19 —	2.65 (2.48)	2.03 (1.99)	4.39 —
7	"	Colorado	26.26 (26.51)	45.54 —	2.51 (2.55)	2.02 (2.01)	4.44 —
8	"	Kansas	26.51	44.93	2.74	2.20	4.90
9	"	Washington	26.09 (26.11)	45.15 —	2.69 (2.52)	2.06 (2.03)	4.56 —
10	1939	?	27.27	44.30	2.44	2.05	4.63
11	"	?	26.73	45.45	2.76	2.10	4.62
12	"	?	25.46	44.15	2.76	2.14	4.85
Maximum			27.73	48.21	3.17	2.23	4.90
Minimum			25.46	43.63	2.44	2.02	4.39
Average			26.61	45.32	2.71	2.11	4.66

\* Results in parentheses are duplicates obtained after original samples had been refrozen following first analysis and are not included in averages.

change on storage over long periods; and (3) that the cholesterol content of the egg solids does not change when the eggs are dried.

#### *Sterol Content of Farinaceous Ingredients of Alimentary Pastes*

Only limited investigations of the quantity of sterols in flour and similar wheat products have been published. Tillmans, Riffart, and Kühn (12) found 0.0102–0.0256 per cent of free sterol calculated as cholesterol on the dry basis in wheat flour and grits. Leulier and Crevat (80) reported 0.039 per cent of sterol in a soft wheat flour. Keding (81) obtained 0.099 per cent of sterol by ether extraction and 0.115 per cent by hydrolysis and then extraction. Riffart and Keller (26) gave the free sterol content on the dry basis in wheat flour and grits as 0.0111–0.0253 per cent calculated as cholesterol. Costa (82) found 0.012–0.015 per cent of free sterol calculated as cholesterol on the dry basis in four Italian semolinas. Hagemann (83) was cited as having found 0.034 per cent of total sterol in hard flour, and Sullivan and Howe (84) obtained 0.018 per cent of total sterol by petroleum benzine extraction of a straight hard wheat flour.

(1) *By the Original Method.*—The original cholesterol method was designed for application to 20 gram samples of alimentary pastes. It was

TABLE 7.—*Cholesterol content of commercial frozen egg yolks*

SAMPLE NO.	YEAR PACKED	SOURCE OF SHELL EGGS (STATE)	TOTAL SOLIDS	FAT BY ACID HYDROLYSIS IN SOLIDS	UNSAPONIFI- ABLE MATTER IN SOLIDS	CHOLESTEROL IN SOLIDS	CHOLESTEROL IN FAT
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	1936	Texas	43.54	59.92	3.59	2.88	4.81
2	"	Neb.-Iowa	45.50	61.54	3.77	2.93	4.76
3	"	California	45.80	61.29	3.55	2.86	4.67
4	"	Kansas	46.07	61.23	4.00	3.15	5.12
			(46.24)*	—	(3.74)	(3.11)	—
5	"	?	44.10	60.61	3.51	2.80	4.62
6	"	Iowa	43.51	61.16	3.70	2.88	4.71
7	"	Mo.-Kansas	40.43	57.98	3.39	2.77	4.78
8	"	California	42.49	59.54	3.49	2.69	4.52
			(42.37)	—	(3.26)	(2.68)	—
9	"	Washington	41.54	58.76	3.39	2.66	4.53
			(41.63)	—	(3.38)	(2.73)	—
10	"	Idaho	42.63	59.10	3.88	3.00	5.08
			(42.69)	—	(3.43)	(2.96)	—
11	"	Colorado	46.10	60.50	3.54	2.86	4.73
12	"	?	44.67	60.53	3.88	2.89	4.77
13	1939	?	44.41	—	3.52	2.95	—
14	"	?	46.11	61.05	3.46	2.96	4.85
15	"	?	45.78	60.35	3.48	2.89	4.79
Maximum			46.11	61.54	4.00	3.15	5.12
Minimum			40.43	57.98	3.39	2.66	4.52
Average			44.18	60.25	3.61	2.88	4.77

\* Results in parentheses are duplicates obtained after original samples had been re-frozen following first analysis and are not included in averages.

TABLE 8.—*Cholesterol content of commercial dried eggs*

SAMPLE NO.	DESCRIPTION OF SAMPLE	YEAR PREPARED	SOURCE OF SHELL EGGS (STATE)	TOTAL SOLIDS	FAT BY ACID HYDROLYSIS IN SOLIDS	UNSAPONIFI- ABLE MATTER IN SOLIDS	CHOLESTEROL IN SOLIDS	CHOLESTEROL IN FAT
				<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	Dried Whole Egg*	1936	Mo.-Kan.	97.94	45.73	2.66	2.20	4.81
2	Dried Yolk†	1936	Mo.-Kan.	97.70	57.83	3.48	2.78	4.81
3	" "	1939	?	97.20	61.56	3.45	2.94	4.78
4	" "	"	?	95.58	60.99	3.48	2.91	4.77
5	" "	"	?	95.59	60.68	3.48	2.84	4.68
Maximum for dried yolk				97.70	61.56	3.48	2.94	4.81
Minimum " " "				95.58	57.83	3.45	2.78	4.68
Average " " "				96.52	60.26	3.47	2.87	4.76

\* Prepared from the same lot of liquid egg as Sample No. 4, Table 6.

† Prepared from the same lot of liquid yolk as Sample No. 7, Table 7.

found that without prior extraction of the fat the unsaponifiable matter could be extracted from such samples after a short acid hydrolysis followed by direct saponification. The unsaponifiable matter was extracted by a procedure based on the modified Kerr-Sorber method. To the unsaponifiable matter was added 20 mg. of cholesterol to provide the excess required as indicated by the coprecipitation studies, and the usual cholesterol dibromide precipitation procedure was applied. The difference between the cholesterol found and that added represents the sterol present in the flour. This procedure and the adsorption modification previously described were applied to the durum flour used in the preparation of the authentic alimentary pastes. By the original method this product contained in the solids 0.13, 0.13 per cent of unsaponifiable matter and 0.037, 0.037 per cent of sterol (as cholesterol); by the adsorption modification, it contained in the solids 0.080 per cent of adsorbed unsaponifiable matter and 0.036 per cent of sterol (as cholesterol).

(2) *By the Revised Method.*—With the development of the oxidative iodometric method, the procedure was revised to apply to 10 grams of flour or alimentary paste instead of the 20 grams previously used.

Samples of the durum flour used in the authentic alimentary pastes and previously analyzed in 1936 had been stored since that time in tightly closed Mason jars at a temperature of about 5° C. The new procedure was applied to this flour in 1940 with the adsorption technic and the sterol was determined by the cholesterol dibromide-sodium iodide method, as well as by the oxidative bromine method, with the following results (dry basis): adsorbed unsaponifiable matter—0.075, 0.075, 0.073, 0.075 per cent; sterol (as cholesterol)—0.031, 0.034 per cent (cholesterol dibromide-sodium iodide method), and 0.031, 0.032 per cent (oxidative bromine method). By the revised method, which does not include the adsorption technic, the results on the dry basis were as follows: unsaponifiable matter—0.12, 0.11 per cent; sterol (as cholesterol)—0.032, 0.030 per cent.

The decrease in the sterol content of this flour between 1936 and 1940 is very slight, but still may be significant, particularly in view of the fact that a decrease in the sterol content of the alimentary pastes of about the same order was observed under similar storage conditions. This decrease is not of practical consequence, however,

The revised method was tested for the recovery of cholesterol added to flour. To 10 gram samples of flour containing 2.7 mg. of sterol (as cholesterol) were added, respectively, 10 mg. and 20 mg. of cholesterol. By the revised method there was recovered, respectively, 9.6 mg. (96 per cent) and 19.4 mg. (97 per cent) of the added cholesterol. The slight amount of cholesterol not recovered is probably lost in the washing out of the soaps from the ether extract of the saponification mixture. It was found that the unsaponifiable matter in the saponification mixture itself is completely extracted by the procedure used.



The revised method was also applied to the determination of the sterol content of samples of flour, durum flour, and semolina actually in use commercially in the manufacture of alimentary pastes. These samples were collected at plants in various parts of the United States by inspectors of the Food and Drug Administration. The results are shown in Table 9.

TABLE 9.—*Sterol content of farinaceous ingredients of commercial alimentary pastes*

SAMPLE NO.	DESCRIPTION OF SAMPLE	TOTAL SOLIDS	UNSAPOINIFIABLE MATTER IN SOLIDS	STEROL (AS CHOLESTEROL) IN SOLIDS	FAT BY ACID HYDROLYSIS IN SOLIDS	STEROL IN FAT
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	Durum Flour	87.95	0.10	0.022	2.22	0.99
2	" "	86.83	0.11	0.023	1.82	1.26
3	" "	87.65	0.11	0.025	2.50	1.00
4	" "	88.59	0.11	0.027	2.47	1.09
5	" "	86.85	0.10	0.022	1.82	1.21
6	" "	89.02	0.10	0.021	—	—
7	" "	88.74	0.12	0.026	2.25	1.16
8	" "	88.94	0.10	0.021	1.93	1.09
9	" "	89.12	0.10	0.021	1.59	1.32
10	" "	88.14	0.12	0.025	2.09	1.20
11	" "	89.64	0.10	0.022	1.84	1.20
12	" "	89.34	0.12	0.031	2.44	1.27
13	Semolina	88.88	0.10	0.021	—	—
14	"	88.61	0.10	0.023	1.81	1.27
15	"	88.23	0.11	0.023	1.43	1.61
16	"	89.67	0.11	0.023	—	—
17	"	89.96	0.10	0.022	2.40	0.92
18	"	87.74	0.10	0.023	1.76	1.31
19	Flour	88.57	0.10	0.025	1.98	1.26
20	"	88.40	0.14	0.044	2.15	2.05
21	"	87.92	0.10	0.024	1.76	1.36
22	"	88.24	0.10	0.025	2.05	1.22
23	"	88.55	0.10	0.028	1.91	1.47
24	"	89.31	0.10	0.021	1.73	1.21
Maximum		89.96	0.14	0.044	2.50	2.05
Minimum		86.83	0.10	0.021	1.43	0.92
Average		88.54	0.11	0.024	2.00	1.26

The values for the fat by acid hydrolysis on these samples are taken from reports by analysts in the field stations of the Food and Drug Administration.

The average sterol content of the farinaceous material is quite low and near the minimum sterol content, hence the probable error involved in applying a correction factor in the calculation of the egg content of alimentary pastes made from farinaceous ingredients of unknown composition is slight.

*Sterol Content of Alimentary Pastes as Index of Egg Content*

In 1908 Popp (43) reported briefly on the possibility of using the Windaus cholesterol dibromide reaction for estimating the egg content of alimentary pastes but gave no data. Cappenberg (85) published a note describing a procedure that involves weighing the cholesterol extracted from 400–500 gram samples of noodles, but also failed to give any data. No further work on this subject appeared until 1930, when Tillmans, Riffart, and Kühn (12) published the micro method previously discussed. Soldi and Testori (86) reported a colorimetric method applicable to approximate estimation of the egg content of alimentary pastes. Riffart and Keller (26) considerably improved the oxidative digitonin micro method as applied to noodles and also devised the colorimetric procedure previously commented upon. Kluge (87) and Costa (82) applied a combination of the sterol and lipid phosphorus determinations to alimentary pastes for detecting adulteration of the latter with plant lecithins.

*Sterol Content and Egg Content of Authentic Alimentary Pastes by Original Method.*—In 1936, H. A. Lepper of the Food and Drug Administration personally supervised the preparation of a series of alimentary pastes by the usual commercial methods. The actual egg solids content of the noodles was calculated from the solids content of the egg products and flour used. The various products were identified by batch numbers. The liquid eggs used were freshly broken, and samples for analysis were frozen for preservation and are so designated in Table 5.

The sterol content of these authentic alimentary pastes was determined in 1936 by the original method and for a few samples by the adsorption modification. The results are shown in Table 10, with the percentages of egg solids found as calculated from the general formula—

$$E = \frac{(S - F) 100}{e - f},$$

in which E = percentage of egg solids in sample (moisture-free basis);

S = percentage of sterol in sample (moisture-free basis);

e = average percentage of cholesterol in egg solids used; and

f = average percentage of sterol in flour used (moisture-free basis).

The small differences in the egg content corresponding to a given sterol content are due to the slight variations in the average sterol values for the eggs and flour used as determined by the respective methods.

*Sterol Content and Egg Content of Authentic Alimentary Pastes by Revised Method.*—The authentic alimentary pastes prepared in 1936 were stored in cardboard cartons in a constant temperature room at 5° C. It was considered inadvisable at this time to go to the necessary expense of preparing a new series of authentic alimentary pastes. However, the revised method was applied to the authentic alimentary pastes prepared in 1936 to test its accuracy in the estimation of the egg content. The re-

TABLE 10.—*Sterol content and egg content of authentic alimentary pastes*  
(Results are on the moisture-free basis)

BATCH NO.	EGGS USED	EGG SOLIDS PERCENT	ORIGINAL METHOD (1936)				REVISED METHOD (1940)			
			UNSATURATED MATTER	STEROL (AS CHOLESTEROL)	EGG SOLIDS FOUND	EGG SOLIDS RECOVERED	UNSATURATED MATTER	STEROL (AS CHOLESTEROL)	EGG SOLIDS FOUND	EGG SOLIDS RECOVERED
			per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	—	0.0	0.13	0.035	0.0	—	0.12	0.029	0.0	—
2	Fresh Yolk	3.1	0.23	0.123	2.9	94	0.22	0.118	3.0	97
3	" "	5.6	0.30	0.197	5.4	96	0.31	0.192	5.5	98
4	" "	10.3	(0.25)	(0.197)	(5.4)	(96)				
5	" "		0.45	0.331	9.9	96	0.47	0.331	10.2	99
6	" "	12.2	(0.39)	(0.330)	(10.0)	(97)				
7	Fresh Whole Egg	5.6	0.50	0.377	11.5	94	0.55	0.406	12.8	105
8	" "	11.3	0.27	0.160	5.5	98	0.27	0.154	5.6	100
9	" "		(0.22)	(0.160)	(5.6)	(100)				
10	" "	5.2	0.39	0.276	10.7	95	0.40	0.267	10.7	95
11	" "		(0.33)	(0.276)	(10.8)	(96)				
12	Fresh Yolk	0.0	0.31	0.191	5.2	100	0.32	0.191	5.5	106
13	" "	0.0	0.14	0.040	0.1	—	0.15	0.040	0.3	—
14	" "	0.0	0.15	0.036	0.0	—	0.13	0.033	0.1	—
15	Chinese Dried Yolk	5.5	0.29	0.186	5.3	96	0.30	0.181	5.4	98
			(0.24)	(0.185)	(5.3)	(96)				

\* Contains added carotene in oil.

† Contains added Vita-Pro (commercial carotene in oil preparation).

The results in parentheses were obtained by the adsorption modification of the original method.

covery of egg solids appears to be somewhat better by the revised method (Table 10).

In an alimentary paste of low egg content the cholesterol may approach the minimum ratio of cholesterol to sitosterol (1:1) required to avoid slight error as shown by the coprecipitation studies. Accordingly provision is made in the method for the addition of 10 mg. of cholesterol to the unsaponifiable matter before the sterol is determined in alimentary pastes containing less than 0.27 per cent of unsaponifiable matter. This is the quantity found in the whole egg noodle (Batch 6), which contains practically the minimum egg content required by the present standard. Batch 2 (3.1 per cent of yolk solids) was found to contain 2.8 per cent of yolk solids by the revised method when cholesterol was not added to the unsaponifiable matter. Hence the omission is not of serious consequence from a practical standpoint.

These results are sufficient to show that the revised method gives a good recovery of the egg solids in alimentary pastes over a wide range of composition. This method does not require reagents that are unduly difficult or expensive to obtain in a satisfactory state of purity, or apparatus not normally available in the food laboratory, and since it is not sensitive to slight variations in conditions and involves no unusual manipulative operations it should give good results in the hands of different analysts.

*Effect of Storage on Sterol Content and Indicated Egg Content of Alimentary Pastes.*—The results given in Table 10 indicate that the sterol content of alimentary pastes does not decrease materially on four years' storage at 5° C. A more severe test was made of the effect of storage on the indicated egg content. The ground samples of noodles prepared for analysis in 1936 were kept in closed Mason jars in the laboratory until 1940, during which time they had been exposed to the high temperatures prevailing in Washington, D. C. for four summers and had developed a strong musty odor. When finally analyzed by the revised method, practically all the sterol originally present in the samples was recovered (Table 11). To calculate the egg content by the general formula the average sterol content of the eggs as determined by the revised method was used, but the maximum original value (0.037 per cent) found in 1936 was taken for the sterol content of the flour. This gives the *minimum* egg solids content on the basis of the sterol. Even under these extreme conditions practically all the egg solids originally present were recovered. It is apparent in both series of analyses made in 1940 that the decrease in sterol content seems to involve chiefly the sterols of the flour.

*Calculation of Egg Content of Commercial Alimentary Pastes from Sterol Content.*—The calculation of the egg content of alimentary pastes from the sterol content requires knowledge as to whether whole egg solids or only yolk solids are present. The tentative method (88) for determining this factor is not entirely satisfactory but will serve until an improved method

TABLE 11.—*Sterol content and minimum indicated egg content of authentic alimentary pastes after 4 years' storage at room temperature*

(Results are on the moisture-free basis)

BATCH NO.	EGGS USED	EGG SOLIDS PERCENT	UNSAPO- NIFIABLE MATTER	STEROL (AS CHOLESTEROL)	EGG SOLIDS FOUND	EGG SOLIDS RECOVERED
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	—	0.0	0.12	0.025	0.0	—
2	Fresh Yolk	3.1	0.22	0.116	2.7	87
3	" "	5.6	0.32	0.190	5.2	93
4	" "	10.3	0.47	0.328	9.9	96
5	" "	12.2	0.53	0.391	12.1	99
6	Fresh Whole Egg	5.6	0.27	0.151	5.2	93
7	" " "	11.3	0.40	0.267	10.5	93
8*	Fresh Yolk	5.2	0.32	0.188	5.1	98
9*	—	0.0	0.14	0.036	0.0	—
10†	—	0.0	0.13	0.029	0.0	—
11	Chinese Dried Yolk	5.5	0.29	0.177	5.1	93

\* Contains added carotene in oil.

† Contains added Vita-Pro (commercial carotene in oil preparation).

is available. Progress has been made toward this end, but an opportunity to complete the work has not yet been afforded. For regulatory purposes it can be concluded from the data presented that the sterol content of an egg noodle is independent of the type of eggs used—fresh, frozen, or dried—and of the time of storage under ordinary conditions.

Pending the accumulation of more data the following formulas are proposed for the calculation of the quantity of commercial egg solids in an alimentary paste:

Per cent of commercial egg yolk solids (moisture-free basis) =

$$\frac{(C-0.024)100}{2.88-0.024} = (C-0.024)35, \text{ and}$$

Per cent of commercial whole egg solids (moisture-free basis) =

$$\frac{(C-0.024)100}{2.11-0.024} = (C-0.024)48, \text{ in which}$$

C = percentage of sterol (calculated as cholesterol) in the sample (moisture-free basis).

## EXPERIMENTAL PART

### *Isolation of Phytosterol Used in Coprecipitation Studies*

The phytosterol was isolated from wheat-germ oil kindly furnished by George S. Jamieson of the United States Department of Agriculture. The procedure used was essentially that described by Anderson, Shriner, and Burr (59). Two preparations had, respectively, melting points of 137°–139° and 138°–139° C.; specific rotations (chloroform, 20°) of –27° and –27.7°; and moisture contents of 2.50 and 3.07

per cent. They showed no significant differences in the coprecipitation studies. By the Anderson-Nabenhauer formula (58) these mixtures are indicated to contain about 15 per cent of dihydrositosterol.

#### *Preparation of Pure Cholesterol Dibromide and Pure Cholesterol*

**Cholesterol Dibromide.**—The procedure is described as applied to 2 grams of cholesterol. The cholesterol in 10 ml. of carbon tetrachloride was cooled in ice until the solution started to congeal. There was added slowly from a buret while the mixture liquefied (but was kept cool) the solution of bromine in carbon tetrachloride (0.3–0.4 gram of bromine per ml., determined by titration) until an excess of 0.2 ml. over the calculated quantity theoretically required was present. The clear solution was cooled in ice water for 15 minutes and 70–80 ml. of petroleum benzine at  $-10^{\circ}$  to  $-15^{\circ}$  C. was added. The mixture was vigorously stirred for about 3 minutes while kept at  $-10^{\circ}$  and filtered rapidly with suction on a Jena G3 filter, and the precipitate was washed with petroleum benzine at about  $-10^{\circ}$  until white. The precipitate was pressed down hard to remove excess solvent, and dry air was drawn through until the filter and precipitate had attained approximately room temperature. The precipitate was broken up, dried to constant weight in a vacuum over phosphorus pentoxide, and kept over phosphorus pentoxide in a desiccator protected from the light. The yield is about 1.5 grams of cholesterol dibromide melting clear at  $114.4^{\circ}$ – $114.8^{\circ}$  C. (corrected) followed by decomposition. This product was found to contain 29.26, 29.26, and 29.29 per cent of bromine in triplicate determinations on macro quantities by ashing with potassium hydroxide and applying the Volhard method. By the oxidative iodometric method on the macro scale quadruplicate determinations indicated 29.12, 29.25, 29.25, and 29.20 per cent of bromine.

**Cholesterol.**—Pure cholesterol was prepared from 13 grams of the pure cholesterol dibromide. The dibromide was dissolved in a liter of acetone at room temperature, 50 grams of sodium iodide was added, and the solution was allowed to stand overnight. The iodine was reduced with a slight excess of sodium thiosulfate solution, and the mixture was diluted to about 2.5 liters with water. The white precipitate was filtered off and washed copiously with water. The product was recrystallized from alcohol (twice) to constant melting point and dried to constant weight over phosphorus pentoxide in an Abderhalden drier with boiling toluene in the heating chamber. The yield was 8.0 grams, melting at  $148.0^{\circ}$ – $148.8^{\circ}$  C. (corrected), corresponding to 87 per cent on the basis of the dibromide used and 63 per cent on the basis of the original cholesterol (15 grams) used in preparing the dibromide.

#### *Unsaponifiable Matter and Cholesterol in Eggs*

##### ORIGINAL METHOD UNSAAPONIFIABLE MATTER

In the extraction of the unsaponifiable matter the original method used did not differ essentially from the revised method described later, but somewhat larger samples were used.

**Adsorption Modification.**—The unsaponifiable matter extracted by the original method was dissolved in 20 ml. of petroleum benzine. There was added 1 gram of  $Al_2O_3$  adsorbent, prepared by igniting basic aluminum acetate powder until white and passing through a 100-mesh sieve, and the mixture was swirled for a minute. The sides of the flask were washed down with a few ml. of petroleum benzine, and the mixture was again swirled. After the  $Al_2O_3$  had settled the petroleum benzine was decanted through a filter tube. The adsorbent was similarly washed with three successive 10 ml. portions of petroleum benzine. The  $Al_2O_3$  with adsorbed matter was then extracted in like manner with ethyl ether (U.S.P.). The ethyl ether extract

was collected in a 25×150 mm. test tube, evaporated, dried, and weighed as directed in the revised method.

#### CHOLESTEROL

The original method did not differ essentially from the revised method to the point where the ice pack is removed from around the filter tubes and the filtrate and washings discarded. Instead a 125 ml. Erlenmeyer flask was placed under the filter, and the precipitate was dissolved out with four successive 10 ml. portions of acetone by the same technic prescribed in the revised method. To the acetone solution was added approximately 0.5 gram of NaI; the flask was stoppered, rotated to dissolve the salt, and set aside in the dark for 1 hour. The iodine was titrated with 0.01 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution to complete disappearance of the yellow iodine color.

mg. of cholesterol =  $1.0 + 2.16 \times (\text{ml. of } 0.01N \text{ Na}_2\text{S}_2\text{O}_3)$ .

#### REVISED METHOD UNSATURIFIABLE MATTER

##### REAGENTS

- (a) *Concentrated potassium hydroxide solution*.—Dissolve 60 grams of KOH in 40 ml. of water.
- (b) *Dilute potassium hydroxide solution*.—Dissolve 11.2 grams of KOH in 1 liter of water.
- (c) *Ether*.—Ethyl ether, U.S.P.
- (d) *Alcohol*.—Approximately 95% ethyl alcohol by volume.
- (e) *Hydrochloric acid*.—(1+4).
- (f) *Dried ether*.—Immediately before use shake U.S.P. ether with an excess of anhydrous CaCl<sub>2</sub> and filter.
- (g) *Anhydrous sodium sulfate*.—Powdered to pass a 60-mesh sieve.
- (h) *Phenolphthalein indicator*.—1% solution in alcohol.

##### APPARATUS

- (1) *Separatory funnel*.—A 500 ml. separatory funnel, ether-tight, with the stop-cock lubricated only with water.
- (2) *Erlenmeyer flasks*.—One 125 ml. and one 300 ml.
- (3) *Filtration bell jar*.—A bell jar of sufficient size to accommodate a 300 ml. Erlenmeyer flask, connected to a vacuum by a 2-way stopcock.
- (4) *Test tubes*.—Two, 25×150 mm. (Includes one to be used as a counterpoise.)
- (5) *Centrifuge bottles*.—Two 8 ounce nursing bottles. (Includes one to be used as a counterpoise.)
- (6) *Pressure siphon*.—A 2-holed rubber stopper to fit the nursing bottles, equipped with a pressure bulb and an adjustable siphon tube of 2 mm. bore.
- (7) *Sintered glass filter*.—A Jena 11G3 sintered-glass filter or its equivalent.

##### DETERMINATION

Weigh accurately into the 125 ml. Erlenmeyer flask approximately 2.5 grams of whole egg, 1.5 grams of yolk, 1 gram of dried whole egg, or 0.7 gram of dried yolk and add 10 ml. of the concentrated KOH solution. Cover with a small watch-glass and heat 3 hours on the steam bath, swirling occasionally to disintegrate any lumps. Cool to about 30°, add 30 ml. of alcohol, and swirl until the insoluble matter is finely dispersed. Add 50 ml. of ether, mix thoroughly, and transfer to the separatory funnel. Wash the flask with two more 50 ml. portions of ether and thoroughly mix the ether solutions by swirling. Wash the saponification flask with 100 ml. of the dilute KOH solution and pour the solution into the separatory funnel in a slow steady stream while swirling the liquid gently. Allow the liquids to separate (about 10 minutes) and slowly draw off the soap solution, including any small quantity of

emulsion, into a nursing bottle. Rinse down the sides of the funnel with 10 ml. of the dilute KOH solution and draw this off into the bottle. Add 50 ml. of ether to the soap solution, close the bottle tightly with a soft moistened cork, and shake vigorously. Centrifuge, and pour the dilute KOH solution in a gentle stream down the side of the bottle until the ether is brought almost up to the neck. Transfer the ether layer to the separatory funnel as completely as possible, using the pressure siphon. Rinse the portion of the siphon tube in the bottle and the walls of the bottle with a 10 ml. portion of ether and transfer the ether to the separatory funnel. Wash the ether solution with successive 100 ml. portions of the dilute KOH solution, swirling the liquid as before, until the washings become only faintly turbid on acidifying with the HCl solution, maintaining the volume of the ether above 150 ml. by addition of more ether if necessary. Wash the ether solution free of alkali with 30 ml. portions of water (test with phenolphthalein). Filter the ether solution into a 300 ml. Erlenmeyer flask with gentle suction through a 3-4 mm. layer of  $\text{Na}_2\text{SO}_4$  on the sintered glass filter, rinsing the separatory funnel and filter with 10, 5, and 5 ml. portions of ether. Rinse the stem of the filter with dried ether, add a porcelain chip to the flask, and evaporate the ether on the steam bath (caution!) to a volume of about 20 ml. Transfer the ether solution to the weighed test tube, using 10, 10, and 5 ml. portions of dried ether. (For satisfactory results the drying and weighing of the tube should be done as follows: Dry the clean test tube and a similar one to be used as a counterpoise at  $100^\circ\text{--}105^\circ\text{C}$ . for 1 hour; remove from the oven and place near the balance with the balance case open for 30 minutes; weigh the tube, using the counterpoise.) Place the tube in a 300 ml. Erlenmeyer flask full of water at an initial temperature of  $45^\circ\text{--}50^\circ\text{C}$ . on the steam bath while directing a stream of clean air upon the surface of the ether so that it is rapidly rotated and evaporate to apparent dryness. Remove the tube, wipe with a clean towel, and place in the oven with the counterpoise at  $100^\circ\text{--}105^\circ$  for 1 hour. Cool, and weigh as before. Deduct from the weight of the unsaponifiable matter any blank obtained from the reagents used, determined by the same procedure.

#### CHOLESTEROL

##### REAGENTS

- (a) *Ice*.—Prepare about 3 gallons of crushed ice.
- (b) *Bromine solution*.—Weigh to 0.1 gram a narrow-mouthed, glass-stoppered, 25 ml. flask containing 5 ml. of  $\text{CCl}_4$ . Add 4-5 grams of Br, weigh again, and dilute with  $\text{CCl}_4$  to a final concentration of  $0.22 \pm 0.02$  gram of Br per ml.
- (c) *Acetic acid solution*.—Pipet 200 ml. of glacial acetic acid into a 250 ml. glass-stoppered, volumetric flask; dilute to the mark with water, mix cautiously, dilute to the mark, and mix again.
- (d) *Asbestos*.—Prepare asbestos as directed under XXXIV, 37, *Methods of Analysis*, A.O.A.C., 1940.
- (e) *Sand*.—Pass clean sand through a 60-mesh sieve and treat with HCl until the extracts are practically colorless. Wash, dry, and ignite.
- (f) *Sodium hypochlorite solution*.—About 1 N in NaOCl and about 0.1 N in NaOH. Dissolve 88 grams of reagent-quality NaOH in 200 ml. of  $\text{H}_2\text{O}$ ; add about 1500 ml. of crushed ice, and pass in Cl until 71 grams is absorbed; dilute to 2 liters and store in dark bottles in the refrigerator. Do not use if the concentration becomes less than 0.95 N in NaOCl.
- (g) *Sodium formate solution*.—Prepare an aqueous solution of reagent-quality sodium formate containing 0.5 gram of the salt per ml.
- (h) *Hydrochloric acid solution*.—Prepare approximately 6 N HCl.
- (i) *Methyl red indicator*.—Dissolve 0.5 gram of methyl red in 50 ml. of 95% alcohol, dilute to 100 ml. with water, and filter.



(j) *Sodium thiosulfate solution*.—0.02 *N*. Prepare from reagent-quality  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  and  $\text{CO}_2$ -free water containing 1% of amyl alcohol. (This solution usually retains its titer for months.) Standardize against an exactly 0.02 *N* solution of pure  $\text{KIO}_3$  as follows: To a glass-stoppered 125 ml. Erlenmeyer flask add 5 ml. of 20%  $\text{KI}$  solution, 10 ml. of water, 2 grams of  $\text{NaHCO}_3$ , and slowly 5 ml. of approximately 6 *N*  $\text{HCl}$ . Mix, add 25–30 ml. of the  $\text{KIO}_3$  solution, wash down the sides of the flask with a few ml. of water, and titrate at once with the thiosulfate solution, using starch solution as indicator.

(k) *Starch solution*.—1% solution of soluble starch.

(l) *Potassium hydroxide solution*.—Dissolve 10 grams of  $\text{KOH}$  in 10 ml. of water.

(m) *Potassium iodide solution*.—20%. This solution must be colorless when acidified with  $\text{HCl}$ .

(n) *Ammonium molybdate solution*.—Dissolve 5 grams of ammonium molybdate in 100 ml. of water.

#### APPARATUS

(1) *Ice bath*.—A container holding about 4 liters and 10–15 cm. deep filled with crushed ice.

(2) *Graduated cylinders*.—One 25 ml. and one 10 ml.

(3) *Test tube*.—18×150 mm.

(4) *Stirring rods*.—Glass rods about 4 mm. in diameter and about 19 cm. long.

(5) *Mohr pipets*.—One graduated to 0.01 ml.; one graduated to 0.1 ml.

(6) *Filtration bell jar*.—A bell jar of sufficient size to accommodate a 300 ml. Erlenmeyer flask, connected to a vacuum by a 2-way stopcock.

(7) *Device for filtering at 0°*.—A filter tube of the Knorr extraction tube style used in the determination of fat in cacao products is needed. This is about 20 mm. in diameter inside, with a body about 11 cm. long and a stem about 10 cm. long. It is provided with the usual perforated disk of nickel or monel metal. Remove the stem at the apex from a 60° Bunsen funnel, 11 cm. in diameter. Enlarge the opening at the apex to about 1 cm. diameter by grinding or grating off the glass. Cut an approximately 1 cm. length from the end of a one-hole rubber stopper of a size that fits snugly in the opening of the funnel. Pass the stem of the filter tube through the stopper in the funnel apex and then through a stopper to fit the bell jar. Prepare in the filter tube a mat of the asbestos 6–8 mm. thick and cover with an approximately 12 mm. layer of the sand.

#### DETERMINATION

Make four determinations at a time if the bell jars are available. Pack the bromine reagent, the 25 ml. graduated cylinder, and the 18×150 mm. test tube containing the four stirring rods in the ice. Pack ice about the filter tubes, taking care none gets into the filters. Cool the acetic acid solution to about  $-5^\circ$  in an ice-salt mixture.

Wash down the sides of the test tubes containing the unsaponifiable matter, while rotating them, with 2.0 ml. of *anhydrous* ether delivered from a Mohr pipet; stopper with a cork and pack the tubes in the ice bath to within about an inch of the top for at least 10 minutes. To one of the tubes add from a Mohr pipet 0.20 ml. of the cold  $\text{Br}$  reagent, mix the contents by swirling, stopper, and replace in the ice bath. Start this procedure at 3 minute intervals with the other tubes. After 10 minutes add rapidly 15 ml. of the acetic acid solution\* measured in the cold 25 ml. cylinder, stir well for 3 minutes, and allow the mixture to stand in the ice bath for 10 minutes. With the suction on, pour the mixture rapidly into the filter tube. Wash down the sides of the test tube with 5 ml. of the cold acetic acid solution and replace in the ice bath. When the liquid in the filter just recedes below the surface of the

\* Caution: This solution is corrosive. If it comes in contact with the hands, rinse them immediately.

sand add the acetic acid from the test tube. Repeat the washing in like manner with 5 ml. of the acetic acid solution and suck the filter free of excess liquid. Wash the test tube and filter with cold water, filling the filter tube about three times. Thoroughly drain the test tube and apply suction to the filter until drops of water cease to fall from the stem. Remove the ice pack from around the filter tube and discard the filtrate and washings. Place a 300 ml. Erlenmeyer flask under the filter so that the stem projects well into the neck of the flask. Wash the test tube and filter with 10 ml. of alcohol, 10, 5, and 5 ml. portions of ether, and finally with 10 ml. of alcohol, gently stirring the sand with each portion of the solvent and allowing the mixture to stand for about a minute before applying the suction. Wash the stem of the filter with a few ml. of ether, add 1 ml. of the KOH solution, mix, and wash down the sides of the flask with 5 ml. of ether. Evaporate the ether and alcohol completely on the steam bath, finally using a stream of clean air to remove the last of the alcohol vapors. Add 40 ml. of hot water to the residual alkaline liquid, mix, and neutralize the alkali with 6 N HCl, using 1 drop of methyl red indicator. Add 10 grams of NaCl, 3 grams of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , and 20 ml. of the NaOCl solution. Bring the solution just to vigorous boiling, remove from the heat, and add immediately, with care, 5 ml. of the sodium formate solution. Cool, and dilute to about 150 ml. with water. Add 5 ml. of the KI solution, a drop or two of the ammonium molybdate solution, and 25 ml. of 6 N HCl. Titrate at once with the  $\text{Na}_2\text{S}_2\text{O}_3$  solution, using starch solution as indicator. Correct the titer for a blank determination on the reagents, starting at the point where the KOH solution is added to the alcohol-ether solution.

$$\text{mg. of cholesterol} = 0.55 + 0.688 \times (\text{ml. of } 0.02 \text{ N Na}_2\text{S}_2\text{O}_3).$$

*Unsaponifiable Matter and Cholesterol in Alimentary Pastes  
and Farinaceous Ingredients*

ORIGINAL METHOD  
UNSAAPONIFIABLE MATTER

The original method for the determination of unsaponifiable matter did not differ essentially from the revised method described below, except that a 20 gram sample was used with appropriately increased quantities of reagents used in the hydrolysis and saponification.

*Adsorption Modification.*—The unsaponifiable matter as extracted by the original method was treated as directed previously under the corresponding method applied to eggs.

CHOLESTEROL

The original method did not differ essentially from the revised method down to the point where the ice pack is removed from around the filter tubes. From this point the procedure follows the original method for cholesterol in eggs.

REVISED METHOD  
UNSAAPONIFIABLE MATTER

REAGENTS

- (a) *Potassium hydroxide.*—Pellets of KOH.
- (b) *Hydrochloric acid.*—(1 + 1).
- (c) *Cholesterol.*—Highest quality cholesterol obtainable, melting point not less than 147°. Test for purity by the revised method.

The other reagents are described under Unsaponifiable Matter in Eggs, with the omission of the concentrated KOH solution.

APPARATUS

The apparatus is described under Unsaponifiable Matter in Eggs, except that the Erlenmeyer flask is replaced by one of 500 ml. capacity.

## DETERMINATION

Weigh a 10 gram sample (ground to pass a 20-mesh sieve) into a 500 ml. Erlenmeyer flask and add with shaking 30 ml. of the HCl. Heat on the steam bath for 30 minutes, shaking the flask occasionally to break up any lumps. While cooling the inclined flask under the tap, add carefully with shaking 30 grams of the KOH pellets at such a rate that the liquid may boil, but not so violently as to cause loss by spurt-ing. While it is still hot, place the flask on the steam bath, cover with a small watch-glass, and heat for 3 hours, with occasional swirling of the mixture to carry down any material adhering to the sides. Cool to about 30° C., add 30 ml. of alcohol and 50 ml. of water, and mix well. Add 100 ml. of ether, swirl the mixture vigorously for a minute, and transfer to the separatory funnel, washing the flask with 50 ml. and 25 ml. portions of ether. Wash the flask with 50 ml. of the KOH solution, pouring this into the funnel in a slow stream while gently rotating the mixture. After the ether layer has separated sharply at the upper liquid interface (about 10 minutes), draw off the lower layers, including any small quantity of emulsion, into a nursing bottle. Proceed as directed under Unsaponifiable Matter in Eggs, beginning with the directions "rinse down the sides of the funnel with 10 ml. of the dilute KOH solution. . . ."

## CHOLESTEROL

The sterol is determined in the unsaponifiable matter as directed for the cholesterol determination in eggs (p. 141). To the unsaponifiable matter from egg-free products or from any containing less than 0.27% unsaponifiable matter (dry basis) add 10 mg. of cholesterol before applying the cholesterol method and correct the result accordingly.

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## SUMMARY

The need for a method for the determination of the egg content of alimentary pastes is discussed.

The factors involved in the adoption of a method based on a cholesterol determination are given, and available methods are considered in the light of such factors.

The conditions necessary for the bromination of cholesterol and precipitation of cholesterol dibromide as the basis of a quantitative determination of cholesterol are presented.

An iodometric method for the determination of cholesterol based on the cholesterol dibromide precipitation and the reaction between cholesterol dibromide and sodium iodide in acetone is given. It was applied to the estimation of the sterol content of eggs, flour, and alimentary pastes.

The interference of wheat phytosterols in the determination of cholesterol by the cholesterol dibromide precipitation method is discussed.

Methods for the preparation of pure cholesterol dibromide and for the purification of cholesterol are described.

An iodometric method for the determination of cholesterol based on the cholesterol dibromide precipitation and the application of the van der Meulen oxidative bromine determination is presented. This method was also applied to the determination of the sterol content of eggs, flour, and alimentary pastes.

It is shown that the sterol content of the solids is not changed by long storage of eggs in the frozen condition or by the commercial process of preparing dried eggs.

Results on the sterol content of a number of samples of commercially prepared frozen eggs and dried eggs and of farinaceous ingredients used commercially in alimentary pastes are given.

The sterol content of alimentary pastes is shown to be a useful index of the egg content.

The fact that the sterol content of alimentary pastes does not decrease materially on long storage at ordinary temperatures is established.

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## AN IMPROVED RAT GROWTH METHOD FOR THE ASSAY OF VITAMIN B<sub>1</sub> INCLUDING SULFITE TREATMENT OF DIETARY CONSTITUENTS\*

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Rat growth methods have long been used for the determination of vitamin B<sub>1</sub>. In the past a major criticism of such methods has been based on lack of specificity of the growth response. A procedure for the assay of vitamin B<sub>1</sub> was described before this Association three years ago by Kline, Tolle, and Nelson (1). It involves the cure of polyneuritis in rats, and thus has as one of its main features a high degree of specificity. This method is now included in the Second Supplement to the U. S. Pharmacopoeia XI. The adequacy of the diet used in the U.S.P. method has been well demonstrated. Assay animals in which polyneuritis has recurred 10 to 20 times have grown to maturity and reproduced normally while being maintained on the U.S.P. diet supplemented with crystalline vitamin B<sub>1</sub>. Further, the administration of relatively large doses of riboflavin, pyridoxin, nicotinic acid, pantothenic acid, or choline, in addition to the usual curative dose of vitamin B<sub>1</sub>, was found to have no influence upon the length of the curative response to vitamin B<sub>1</sub>.

The curative method is particularly useful in the assay of relatively rich sources of vitamin B<sub>1</sub>. However, when used for the assay of food products and materials that may be considered of low vitamin B<sub>1</sub> potency, difficul-

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ties of administering large test doses to the polyneuritic animal must be considered. For the assay of such substances the addition of the test material to the diet and comparison of increases in body weight of animals fed reference standard and test material are most practical. The accuracy of such a procedure is largely dependent upon the adequacy of the diet used.

In the preparation of dietary materials free from vitamin B<sub>1</sub> the use of sulfite for the destruction of vitamin B<sub>1</sub> has a practical application. Details of this procedure have been studied for some time in this laboratory. Preliminary reports of these investigations by Kline, Tolle, and Nelson (2) and by the Associate Referee for Vitamin B<sub>1</sub> (3) (4) have appeared recently. These studies will be more fully described at this time.

#### SULFITE STUDIES

The sulfite reaction was first applied to yeast by treating for 5 days, 50 grams of this material in 500 ml. of 0.1 per cent sodium sulfite, with sulfur dioxide added to bring the pH to 4.0. Under these conditions vitamin B<sub>1</sub> was found to be completely destroyed, and there was no evidence of loss of any other dietary essential. This process, however, involved the disagreeable use of sulfur dioxide gas and a long period of treatment. Further study of the conditions most favorable to the sulfite reaction are summarized in Table 1. In this study of the effect of varying pH, crystalline thiamine was dissolved in 0.05 per cent sodium bisulfite then buffered at different pH values from 2.7 to 7.1. The resulting solutions were left to stand for 4 days. In Table 1 it is indicated that destruction of vitamin B<sub>1</sub> as measured biologically was complete at pH values from 3.2 to 6.0. Above and below these values the destruction was incomplete and occurred to an extent varying from 25 per cent to 75 per cent. Such results may indicate that the bisulfite ion is the active factor in the vitamin B<sub>1</sub> destruction since at the higher pH value sulfite presumably would be present, and at the lower pH, sulfur dioxide. On this basis the pH of the bisulfite, 4.5, would be expected to be the most effective in destroying the vitamin.

A second point studied was the effect of period of treatment. It was found that with materials in which the vitamin B<sub>1</sub> was in solution the time necessary for completion of the splitting reaction was relatively short. When yeast extract was treated with sodium bisulfite at pH 5.3 the destruction was incomplete in 20 minutes but complete in 45 minutes. At pH 7.1 the destruction was incomplete at 45 minutes, again indicating the effect of pH. When rice polish extract was used the destruction was complete in 1.5 hours at pH 4.5 but incomplete in the same period of time at pH 7.3. With wheat germ treated at pH 5.3 the destruction was complete in 2 hours. With liver extract destruction was found to be incomplete in 2 hours but complete in 24 hours. The 24 hour period is con-

TABLE 1.—*Factors affecting the sulfite destruction of vitamin B<sub>1</sub>*

SAMPLE		CONC. NaHSO <sub>3</sub>	TIME	pH	RESULTS
		per cent	days		
1. Effect of pH	Crystalline Thiamin	0.05	4	2.7	Incomplete
				3.2	Complete
				6.0	
				6.5	Incomplete
				7.1	
			minutes		
2. Effect of Time	Yeast Extract	1.23	20	5.3	Incomplete
		"	45	5.3	Complete
		"	45	7.1	Incomplete
	Rice Polish Extract	1.23	hours		
			1.5	4.5	Complete
		"	1.5	7.3	Incomplete
	Wheat Germ	0.42	2	5.3	Complete
	Liver Extract	0.6	2	4.5	Incomplete
3. Effect of Con- centration of NaHSO <sub>3</sub>	Liver Extract	"	24	4.5	Complete
		0.064	2	No excess SO <sub>2</sub>	
		0.123	2	" " "	
		0.6	2	Excess SO <sub>2</sub>	

sidered safe and practical, and is now being used in the routine preparation of dietary constituents.

In the study of concentration of bisulfite necessary for the reaction, there seemed to be no direct relationship between the bisulfite needed and the quantity of vitamin B<sub>1</sub> to be destroyed. The concentration depended rather upon the character of the material treated. Of the materials studied, liver extract was found to require the greatest concentration. This may be due to the presence of factors that render the bisulfite inactive by oxidation to sulfate. It is also known that animal tissues have a bisulfite binding capacity. Liver extract in a solution of sodium bisulfite in a concentration of 0.064 per cent showed no excess sulfur dioxide upon being acidified after standing at room temperature for 2 hours. This was true also of a concentration of 0.123 per cent. However, when 0.6 per cent solution was used, containing 1 part of the dry liver extract in 10 parts of solution, the presence of excess sulfur dioxide upon acidification indicated that there was a sufficient quantity for the desired result.

In Table 2 are indicated the steps in the treatment of two dietary constituents representing water-insoluble and water-soluble types of material. If crude casein is to be used it must be washed free of excess acid with 1 or 2 washings in large volumes of water. For this purpose 2 kg. of casein in 20-gallon volumes of water was used. After being washed, this material was suspended in 10 times its weight, or 20 liters, of 0.6 per cent sodium bisulfite in a stoppered bottle. It was then left to stand at room temperature for 24 hours, but was stirred or agitated 2 or 3 times during this period. The supernatant liquid was then siphoned off, and the sulfite was



TABLE 2.—*Details of sulfite treatment*

CRUDE CASEIN	LIVER EXTRACT
1. Wash to pH 4.5	1. Dissolve 100 grams in 1 liter of 0.6% NaHSO <sub>3</sub> .
2. Suspend 2 kg. in 20 liters 0.6% NaHSO <sub>3</sub> in stoppered bottle.	2. Adjust to pH 4.5.
3. Let stand 24 hours, room temperature. Stir occasionally.	3. Let stand 24 hours in stoppered flask, room temperature.
4. Wash in large volume of water.	4. Make acid with HCl to pH 1.5 and reduce to $\frac{1}{2}$ volume in vacuum still (50°C).
5. Dry at 60°C.	5. Neutralize (NaOH) and dry (50°C) on requisite amount of treated casein.

washed from the casein with a large volume of water. The material was then dried at 60° C. In the case of liver extract, 100 grams was dissolved in one liter of 0.6 per cent sodium bisulfite, and pH was adjusted to 4.5 with hydrochloric acid. This material was left to stand for 24 hours at room temperature in a closed flask. After acidification to pH 1.5 with hydrochloric acid its volume was reduced by approximately one-half in a reduced pressure still. The temperature in this procedure should not exceed 50° C. The liver extract solution was then neutralized with sodium hydroxide and dried on the requisite quantity of treated casein at a temperature not exceeding 50° C. These treated materials were then ready for inclusion in the diet.

#### GROWTH METHOD OF ASSAY FOR VITAMIN B<sub>1</sub>

The assay, comprising the recording of observations of rats throughout specified periods of their lives while being maintained on specified dietary regimens and the interpretation of such data, is as follows:

##### PRELIMINARY PERIOD

Throughout the preliminary period each rat shall be raised under the immediate supervision of, or according to directions specified by, the assayer. Throughout the preliminary period the rats shall be maintained on a dietary regimen that shall provide for normal development in all respects, except that such dietary regimen shall subsequently allow in rats weighing between 40 and 50 grams and not exceeding 26 days of age and subsisting on a suitable vitamin B<sub>1</sub>-deficient diet and water for an interval not exceeding 50 days, the development of characteristic symptoms of vitamin B<sub>1</sub> deficiency (polyneuritis).

##### DEPLETION PERIOD

A rat shall be suitable for the depletion period when the age of the rat does not exceed 28 days, and if the body weight of the rat exceeds 39 grams and does not exceed 50 grams, and if the animal manifests no evidence of injury, or disease, or anatomical abnormality that might hinder growth or development. Throughout the depletion period each rat shall be provided with the vitamin B<sub>1</sub> test diet and water *ad libitum*, and during this period no other dietary supplement shall be available to the animal. Throughout the depletion period and until the assay shall have been completed the rats shall be kept in cages provided with a wire cloth bottom, each mesh of which shall be not less than 8 mm. by 8 mm.

## ASSEMBLING RATS INTO GROUPS FOR THE ASSAY PERIOD

Rats that are suitable for the assay period shall be assembled into groups of at least 8 animals. For each assay material there shall be one or more assay groups. In the assay of one assay material there shall be provided at least one control group and at least one reference group, but one control group and one reference group may be used for the concurrent assay of more than one assay material. The interval of assembling rats into groups shall not exceed 21 days. On any one day during the interval of assembling rats into groups the total number of rats that shall have been assigned to make up any one group shall not exceed by more than two the number of rats that shall have been assigned to make up any other group. When the assembling of all groups shall have been completed the total number of rats in each group shall be the same, and the number of rats of one sex in each group shall be the same. Not more than 3 rats from one litter shall be assigned to one group. When the assembling of all groups shall have been completed, the average weight of the rats in any one group on the day beginning the assay period shall not exceed by more than 10 grams the average weight of the rats in any other group on the day beginning the assay.

## ASSAY PERIOD

A rat shall be suitable for the assay period provided the depletion period shall have exceeded 10 days and shall not have exceeded 30 days, and provided that the rat shall manifest evidence of vitamin B<sub>1</sub> deficiency characterized by stationary or declining weight. Throughout the assay period each rat of the control, reference, and assay groups shall be kept in an individual cage and shall be provided with vitamin B<sub>1</sub> test diet and water *ad libitum*. Throughout the assay, reference material shall be administered to each rat in the reference group and the assay material shall be administered to each rat in the assay group. The assayer shall determine the method of administering the reference and assay material, but in any one assay the reference and assay material shall be administered in the same manner. During the assay period all conditions of environment shall be maintained as uniformly as possible with respect to the assay, reference, and control groups.

## RECORDING OF DATA

On the day beginning the depletion period there shall be a record made of the body weight of each rat. From the 7th day of the depletion period until the beginning of the assay period a record shall be made of the body weight of each rat at intervals not exceeding 3 days. During the assay period a record shall be made of the body weight of each rat at intervals not exceeding 7 days.

VITAMIN B<sub>1</sub> POTENCY OF THE ASSAY MATERIAL

In determining the vitamin B<sub>1</sub> potency of the assay material the performance of the rats of the assay and reference groups shall be calculated on the basis of the difference between the average weight of each group at the end of the assay period and the average weight of the same rats on the day beginning the assay period. The data from the reference group shall be considered valid for establishing the vitamin B<sub>1</sub> potency of the assay material only when two-thirds or more of the total number of animals comprising a reference group shall have made individually, between the beginning day of the assay period and the 28th day thereafter, an increase in body weight that shall equal or exceed 12 grams and shall not exceed 60 grams. The data for an assay group shall not be valid for establishing the vitamin B<sub>1</sub> potency of an assay material if the average weight of the control group is greater at the end of the assay period than at the beginning of the assay period.

The total amount of the assay material administered during the assay period

contains a quantity of vitamin B<sub>1</sub> equal to or greater than the total amount of vitamin B<sub>1</sub> administered to the reference group during the assay period if that quantity promotes in the assay group an average gain in weight equal to or greater than the average gain in weight produced in the reference group by administration of the reference material.

If the reference material and assay material are administered by inclusion in the diet (option 4) then comparison of the quantities of reference and assay material shall be made on the basis of the quantity of each contained in 100 grams of the supplement diets.

#### DEFINITIONS

As herein used, unless the context otherwise indicates, the term *administer* means to supply the reference material or assay material to each rat in any one of the following manners:

(1) By injecting parenterally; (2) by placing in mouth of animal daily; (3) by placing before animal daily in dish separate from vitamin B<sub>1</sub> test diet; and (4) by replacing an equal weight of sucrose in the vitamin B<sub>1</sub> test diet and intimately mixing the material with the diet.

The term *assayer* means the individual immediately responsible for the interpretation of the assay; the term *assay group* means a group of rats to which the assay material shall be administered during the assay period; the term *assay material* means the material under examination for its vitamin B<sub>1</sub> content; the term *assay period* means the interval in the life of a rat between the last day of the depletion period and the 29th day thereafter; the term *assemble* means the procedure by which rats are selected and assigned to groups for the purpose of feeding, care and observation; the term *control group* means a group of rats receiving no assay or reference material during the assay period; the term *daily* means 6 days of each week of the assay period; the term *depletion period* means the interval in the life of a rat between the last day of the preliminary period and the first day of the assay period; the term *reference group* means a group of rats receiving the reference material during the assay period; the term *reference material* means the U.S.P. Vitamin B<sub>1</sub> Reference Standard; the term *stationary or declining weight* means the condition of a rat when the body weight of the rat on any given day is equal to or less than the body weight of the rat on the 5th day prior to the given day; the term *vitamin B<sub>1</sub> test diet* means a uniform mixture that has not been compounded for more than 14 days of the following food materials and in the proportions designated.

#### Vitamin B<sub>1</sub> Test Diet

	per cent
Sucrose	60
Casein (1)	18
Salt Mixture (2)	4
Autoclaved Yeast (3)	5
Autoclaved Peanuts (4)	10
Treated Liver Extract (5)	1
Cod Liver Oil (U.S.P.)	2

(1) Free from demonstrable traces of vitamin B<sub>1</sub>.

(2) Either salt mixture No. 1, described on page 133 of the Second Supplement to U. S. Pharmacopoeia XI, or a salt mixture having essentially the same proportions of the elements.

(3) Prepared by autoclaving in steam at 15 pounds pressure for 5 hours layers of dried yeast not more than 6 mm. in depth and then drying at a temperature not exceeding 65° C.

(4) Prepared by crushing unroasted, shelled, No. 1 Virginia peanuts in a food chopper; autoclaving in steam at 15 pounds pressure in layers not more than 12 mm. in depth; then drying at a temperature not exceeding 65° C. Incorporated in the basal diet by grinding with the requisite quantity of sucrose.

(5) Prepared by dissolving 100 grams of extract of liver in 1 liter of 0.6% NaHSO<sub>4</sub>. The salt used should be tested to insure bisulfite content. This solution is allowed to stand 24 hours in a well-stoppered bottle; then acidified with HCl to pH 1.5; distilled under reduced pressure at a temperature not exceeding 50°C. until the volume has been reduced to one-half the original volume; and finally dried on vitamin B<sub>1</sub>-free casein at a temperature not exceeding 65° C.

### RESULTS

That the diet used in this assay method is complete in all respects except for vitamin B<sub>1</sub> was demonstrated by maintaining rats on the basal diet supplemented with crystalline vitamin B<sub>1</sub> from the period of weaning through the stages of maturity and reproduction. Such animals grew normally from time of weaning to maturity and were capable as well of normal reproduction.

TABLE 3.—*Vitamin B<sub>1</sub> assay results*

ASSAY NO.	ASSAY PERIOD	RAT GAIN PER WEEK (GRAMS) ON INDICATED QUANTITIES OF VITAMIN B <sub>1</sub> PER 100 GRAMS OF DIET (MICROGRAMS)					
		40	50	60	70	80	100
	<i>week</i>						
1	2					19.2	23.8
2	2	1.0		7.5		15.6	
	3	Failure		8.1		15.9	
	4	Failure		8.2		16.4	
3	2			11.1		19.0	
4	2		11.0	10.2	12.7	16.8	
	3		9.0	10.6	13.6	17.5	
	4		7.3	9.1	13.4	17.9	

The data presented in Table 3 indicate the uniformity that may be expected in the use of this method. They are taken from the results of four separate experiments and show the average gains in body weight for groups of rats fed the graded quantities of the reference standard for vitamin B<sub>1</sub>. In Experiments 2 and 4 the average weight increases are given for 2, 3 and 4 week periods of the assay. Except for the 50 microgram level, the results at the end of the second week are as uniform as those at the end of the fourth week, which offers the possibility of using a shorter assay period. However, the longer period is preferred until more experience with the method has been had. Differences of only 10 micrograms per 100 grams of diet are easily differentiated by this procedure, and presumably the sensitivity would be increased by increasing the number of animals in each of the assay groups.

## SUMMARY

Results of studies of the conditions under which sulfite is most effective in the preparation of vitamin B<sub>1</sub>-free dietary constituents are described. It was found that the use of 0.6 per cent sodium bisulfite in the relation of 10 parts of solution to 1 part of material to be treated, at pH 4.5, for 24 hours is a safe and practical method of treatment.

The rat-growth procedure for vitamin B<sub>1</sub> assay described includes the use of a diet containing sulfite-treated casein and sulfite-treated liver extract. This method has been found to have a good degree of sensitivity.

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## DETERMINATION OF GLYCOGEN IN OYSTERS

By H. N. CALDERWOOD\* and ALFRED R. ARMSTRONG (Fish and Wildlife Service, U. S. Department of the Interior, Virginia Commission of Fisheries, and The College of William and Mary in Virginia)

During the digestion of many samples of oyster (*Ostrea virginica*) tissue by the A.O.A.C. procedure (1) for the isolation of glycogen, parts of the ground fresh tissue were not disintegrated after two hours of heating, despite occasional stirring and pressing with a mushroomed stirring rod. Frequently the glycogen precipitated, upon the addition of alcohol, in the form of sticky lumps, which not only retarded washing and filtering of the precipitate but also gave it a grey color.

Although the literature showed several variations in the alkaline method for the isolation of glycogen from animal tissue, it contained no explanation of the above difficulties. When Pfüger first described (2) the separation of glycogen by digestion of the tissue with potassium hydroxide and precipitation of the glycogen from the alkaline solution by the addition of alcohol, he used 60 per cent potassium hydroxide and a heating period of 3 hours. In a subsequent paper (3) he reported that 30 per cent potassium hydroxide and heating 30 minutes gave, with both muscle and liver tissue, results fully as satisfactory as those obtained by the stronger alkali and the longer heating. Although Pfüger's studies were confirmed by later workers (4) a recent paper (5) on the determination of glycogen in oysters states that digestion for 3 hours in a boiling water bath with 56-60 per cent potassium hydroxide is necessary.

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TABLE 1.—*Study of conditions for alkaline (60% NaOH) isolation and alcoholic precipitation of glycogen from oyster tissue*

SAMPLE NUMBER	NaOH ml.	HEAT USED	DILUTION CONDITIONS	ALCOHOL PRECIPITATION CONDITIONS			APPEARANCE OF PRECIPITATED GLYCOGEN—	
				LYE TEMP.	ALCO. TEMP.	RATE	STERED	AFTER 0.5 OR 1.0 HOURS
1	50	HP	HL, IW	IS	IA	R	No	Soln clear after 20 hrs.
2	50	WB	CL, CW	CS	CA	R	No	Fluffy, settled rapidly
3	25	HP	CL, CW	WS	WA	S	Yes	Soln clear after 20 hrs.
4	25	WB	CL, CW	IS	IA	R	No	Soln very clear at 1 hr.
5	50	HP	CL, CW	CS	IA	S	Yes	Fluffy, settled rapidly
6	50	WB	HL, IW	CS	CA	S	Yes	Soln turbid after 20 hrs.
7	25	HP	HL, IW	IS	IA	R	No	Soln clear after 20 hrs.
8	25	WB	CL, CW	WS	WA	S	Yes	Soln turbid after 1 hr.
9	50	HP	CL, CW	WS	WA	R	No	Soln very clear at 20 hrs.
10	50	WB	CL, CW	IS	IA	S	Yes	Soln al. turbid after 20 hrs.
11	25	HP	CL, CW	CS	CA—	S	Yes	Soln clear after 20 hrs.
12	25	WB	HL, IW	IS	IA	R	No	Cloudy after 1 hr.
								Sl. turbid after 20 hrs.
								Soln clear after 20 hrs.

<sup>1</sup> Precipitate became fluffy after washing.

Explanation of symbols: HP, electric hot plate, temp. of soln on plate, 113°–108° C.; WB, steam-heated water bath, temp. of soln in beaker set into bath, 80° C. HL, hot lye; CL, cool lye, at room temp.; CW, cool water, at room temp.; IW, ice water; IA, alcohol at 0° C.; IS, diluted lye at 0° C.; CS, diluted lye at room temp.; WS, diluted lye at 80° C.; CA, alcohol at room temp. 30°–35° C.; WA, alcohol at 60° C.

## EXPERIMENTAL

*Digestion with Alkali.*—The experimental work reported here was conducted in order to surmount the difficulties enumerated in the first paragraph of this paper. With but two exceptions all analyses were made upon freshly ground tissue from oysters that were shelled immediately before being ground. A uniform procedure was used for shelling and draining the oysters.

The first factors studied were the effect of the quantity of lye, and of the temperatures of digestion, dilution, and precipitation on the physical character of the precipitated glycogen. These data are presented in Table 1.

After digestion every sample contained unbroken particles of tissue. In general, the samples heated on the electric hot plate contained only a few, while those heated on the water bath usually had many unbroken particles at the end of the 2 hour period. In every case, after dilution of the lye, these particles were readily disintegrated by crushing and stirring. The temperatures reported were measured with the thermometer in the alkaline liquid. The results obtained (Table 1) show that when oyster tissue is digested with 60 per cent sodium hydroxide a white fluffy precipitate of glycogen is obtained by the following conditions: Either 25 or 50 ml. of 60 per cent lye per 10 grams of ground tissue; digestion made at 80° C. on the water bath; the alkaline hydrolyzate diluted and cooled; and the glycogen precipitated by the rapid addition of cool alcohol with as little stirring as possible consistent with proper mixing of the two liquids. During these experiments it was noticed that precipitates of glycogen allowed to stand more than 24 hours gradually lost their fluffy character and became difficult to filter.

Since temperature was found to be the factor controlling the gummy character of the precipitated glycogen, studies were made on the effects of digestion for a fixed time at different temperatures, and at a fixed temperature for varying periods of time, on the physical characteristics of the precipitated glycogen. These results are reported in Tables 2 and 3.

A 10 gram portion of the ground tissue was used for each of the samples reported in Tables 2 and 3. These samples were digested with 25 ml. of 60 per cent sodium hydroxide for the periods shown in the tables. After digestion the sample was diluted with 125 ml. of water, all lumps were crushed with a stirring rod, and 150 ml. of alcohol was added. After the mixture had stood for 24 hours the supernatant liquid was poured off, and the glycogen precipitate was washed once by decantation with 100 ml. of 66 per cent alcohol and then transferred, by means of 66 per cent alcohol, to a graduated cylinder. Each cylinder was filled to the mark (100 ml.) with 66 per cent alcohol, and 24 hours later the volume of the precipitated glycogen was read.

The data obtained (Tables 2 and 3) indicate that digestion with 60 per cent sodium hydroxide for a short period of time is the most satis-

TABLE 2.—*Effect of digestion temperature on physical condition of glycogen precipitate*  
(All digestions were for 2 hours)

SAMPLE NO.	TEMP.*	HEAT	SETTLED GLYCOGEN		TEMP. RANGE
			VOLUME	COLOR	
13	100	HP	ml. 4.5	Tan	°C. ±3
14	90	HP	6	"	"
15	80	WB	7	Light tan	+1
16	70	WB	7.5	Very light tan	"
17	56	HP	9	White	+2

\* Actual temp. of digesting mixture.

Explanation of symbols: HP, electric hot plate; WB, steam heated water bath

factory for obtaining a glycogen in the best physical condition for purification.

After this qualitative work on the factors influencing the physical characteristics of the glycogen, studies were undertaken to ascertain whether the various conditions of digestion had any effect on the quantity of glycogen obtained. The data in Tables 4, 5, and 6 summarize the results of these studies. Throughout this paper the glycogen content is reported on the basis of the wet ground tissue. Moisture determinations were not made on the several lots of tissue used for this work.

In all quantitative experiments filtrations were made with 18.5 cm. Whatman No. 54 grade filter paper, which was found to be far superior to any other paper that could be obtained. This paper permits rapid filtration, suffers but little deterioration from the strongly alkaline 50 per cent alcohol solution, and almost always gives brilliant filtrates. Except in a few cases it also rapidly filtered the solution of redissolved glycogen.

Examination of Table 4 reveals that higher temperatures, prolonged heating even at lower temperatures, and higher alkali concentrations yield glycogen precipitates that are more difficult to manipulate in the subsequent operations. Samples 25, 27, and especially 32 show how appreciable

TABLE 3.—*Effect of digestion time on physical condition of the glycogen precipitate*  
(All samples were digested at 80° C. ±2°)

SAMPLE NO.	DIGESTION TIME	SETTLED GLYCOGEN	
		VOLUME	COLOR
	hours	ml.	
18	$\frac{1}{2}$	8.5	White
19	$\frac{1}{2}$	7	"
20	1	7	"
15	2	7	Light tan
21	6	7	" "
22	23	8.5	Tan



losses of glycogen can occur by digestion at higher temperatures. This study included 50 per cent of sodium hydroxide, which readily precipitates sodium carbonate and is fluid at room temperature, whereas the 60 per cent solution, being a solid at room temperature, must be heated for precipitation of the carbonate and for use.

Table 5 was planned as a continuation of Table 4 and immediately after the samples listed in the latter had been weighed the ground tissue was placed in a refrigerating chamber, the maximum temperature of

TABLE 4.—*Effect of digestion time and temperature on glycogen recovered from oyster tissue*

SAMPLE NO.	NaOH	HEATING		PRECIPITATE		SAMPLE WEIGHT	GLYCOGEN	REMARKS
		TIME	TEMP.	VOLUME	COLOR			
	percent	hours	0°C.	ml.		grams	per cent	
23	50	2	106	6.5	Tan	10.08	2.06	
24	50	1	80	7	White	10.14	2.00	
25	50	2	80	7	"	10.00	2.07	0.02% glycogen recovered from residue in filter.
26	50	27	80	6	Light tan	10.04	1.75	Total glycogen 2.09%.
27	60	27	80	8	" "	10.05	1.77	0.04% glycogen recovered from residue in filter.
28	60	27	80	8	" "	10.00	1.82	Total glycogen 1.81%.
29	60	2	80	6.5	" "	9.98	1.87	
30	60	2	80	9	" "	10.11	1.91	
31	60	2	106	6.5	Tan	10.05	1.92	
32	60	2	120	7	"	10.11	1.80	0.13% glycogen recovered from residue in filter.
								Total glycogen 1.93%.

NOTES: All samples were washed four times with 66% alcohol (400 ml.), then transferred to 100 ml. cylinders. Color and volume were read after samples had stood for 24 hours.

Samples 23, 26, 28, 31, and 32 filtered more slowly than the others. Samples 26 and 28 gave cloudy filtrates. Residues on filters from Samples 25, 27, and 32 were washed into 100 ml. volumetric flasks and hydrolyzed with 5 ml. of conc. HCl at 90° C. for 3 hours, and glycogen was determined in an aliquot. Sample 32 had a large residue on the filter. Average time to filter original sample and its first wash was 10-15 minutes. Each sample was treated with 25 ml. of the NaOH of the stated concentration.

which never exceeded  $-1.11^{\circ}$  C. ( $30^{\circ}$  F.), for 11 days. The deterioration that occurred under these conditions is apparent in the lower glycogen content of the samples. An extensive study of this question is contemplated.

In view of the difficulty reported in the literature (6) of isolating a nitrogen-free glycogen precipitate, attempts were made to determine the quantity of nitrogen in the precipitated glycogens. In the first trial one-fifth aliquots of Samples 25, 27, and 32 were used. In the second trial four-fifths aliquots of Samples 35, 37, 39, and 41 were used, but unfortunately the color developed on nesslerization by the reagent blanks was so dark that no distinction could be made between the samples and the blank. All the aliquots were treated by the Kjeldahl method, Hengar

TABLE 5.—*Effect of digestion temperature and lye concentration on glycogen recovered from oyster tissue*

(All digestions were for 2 hours with 25 ml. of lye for each sample.)

SAMPLE NO.	NaOH	TEMP.	SAMPLE WEIGHT	GLYCOGEN
	<i>per cent</i>	<i>°C.</i>	<i>grams</i>	<i>per cent</i>
33	50	60	10.31	1.40*
34	50	80	10.22	1.22
35	50	80	9.97	1.29
36	50	60	9.98	1.29
37	50	60	9.99	1.26
38	60	80	10.11	1.31
39	60	80	10.14	1.23
40	60	60	10.32	1.33
41	60	60	10.06	1.36

\* Sample 33 was weighed out immediately after the ground tissue had thawed completely, and the result is not comparable with the others. All other samples were weighed 2 hours later.

Samples 34, 35, 38, and 39 gave slimy precipitates that were light tan after being washed.

Samples 33, 36, and 37 were moderately fluffy with white precipitates after being washed.

selenized granules being used as the catalyst. The color was traced to ammonium salts in the sulfuric acid used.

The observation that particles that resisted 60 per cent lye dissolved readily when the lye was diluted, together with the favorable reports in the literature (3) (4) on the use of 30 per cent potassium hydroxide for the isolation of glycogen from muscle and liver tissue prompted the study of 30 per cent sodium hydroxide for use with oyster tissue. Table 6 summarizes this work, which was done upon fresh animals of the size used as seed for planting commercial oyster beds. All results fall within the limits of error of the analytical method used. Samples 48 and 49 of this lot of tissue were, after the second precipitation, analyzed for nitrogen by the procedure previously described. Both precipitate and filter being digested, an entirely new supply of reagents was used for these analyses, but, again, there was no appreciable difference, upon nesslerization, between the samples and the blank of the reagents. The amount of nitrogen present was estimated to correspond to about 1 mg. per gram of glycogen.

TABLE 6.—*Effect of digestion temperature and lye concentration on glycogen recovered from oyster tissue*

(All digestions were for 2 hours)

SAMPLE NO.	NaOH	TEMP.	SAMPLE WEIGHT	GLYCOGEN
	<i>per cent</i>	<i>°C.</i>	<i>grams</i>	<i>per cent</i>
42	30	80	10.68	1.05
43	30	80	10.30	1.05
44	50	80	10.25	1.08
45	50	80	10.18	1.06
46	50	80	10.15	1.05
47	50	80	10.10	1.07

Encouraged by the satisfactory results obtained with 30 per cent sodium hydroxide (Table 6), the writers made further experiments with this concentration of alkali. The factors studied were time of digestion, size of sample, and effect of a single precipitation upon the quantity of glycogen obtained. These experiments were made upon three different lots of fresh tissue, one lot from oysters that were not "fat,"\* (Table 8); another lot from oysters of medium "fatness" (Table 7); and a third lot from some that were very "fat" (Table 9).

An examination of the results in Table 7 shows that digestion at 80° for one hour with 30 per cent sodium hydroxide is sufficient to liberate all the glycogen from ground oyster tissue. Although the glycogen precipi-

TABLE 7.—*Effect of time of digestion and number of precipitations on glycogen recovered from oyster tissue*

(All digestions were made with the temperature of the lye at 80° ± 2°. All samples were treated with 25 ml. of lye)

SAMPLE NO.	NaOH	TIME	PPTS	SAMPLE WEIGHT	GLYCOGEN	REMARKS
	<i>per cent</i>	<i>hours</i>		<i>grams</i>	<i>per cent</i>	
49	50	2	2	10.08	4.45	Samples 49-52 had white fluffy first precipitates.
50	50	2	2	10.11	4.56	
51	50	1	1	10.30	4.33	
52	50	1	1	10.02	4.45	First precipitates of Samples 53-58 were light grey and not as fluffy as those of 49-52. The former precipitates never lost their grey tinge when washed.
53	30	1	2	10.20	4.46	
54	30	1	2	10.21	4.55	
55	30	1	1	10.22	4.67	
56	30	1	1	10.03	4.48	Samples 57 and 58 gave very turbid water solutions and had much sediment after hydrolysis.
57	30	$\frac{1}{2}$	1	10.10	4.36	
58	30	$\frac{1}{2}$	1	10.19	4.40	

Samples 49-53 leached very slowly with hot water.

tates isolated with 30 per cent sodium hydroxide were light grey† and not so fluffy as those from the 50 per cent alkali, all of the former filtered satisfactorily, leached more rapidly from the filters with hot water, and gave acceptable values. The 15 minute digestions with 30 per cent sodium hydroxide gave glycogen precipitates that yielded final glycogen values fully within the limits of error for the method, despite their grey color after washing (7), their very turbid water solutions, and their deposition of much sediment after acid hydrolysis. This shows that for oyster tissue the use of a prolonged time of digestion, a lye concentration greater than

\* The term "fat" as applied to oysters refers to their glycogen content, and an experienced observer can readily judge this from the color and turgor of the animal when shelled.

† These oysters were taken directly from an oyster boat. They had been collected from muddy beds and there was considerable mud entrapped in the mantle tissue. This mud was not removed by rinsing the shelled carcasses, and it accounts, in part at least, for the grey color of the glycogen precipitates. After the glycogen had been leached from the filter with hot water the mud remained on the filter as a black residue.

TABLE 8.—*Effect of digestion at 80°C. for varied periods of time at different lye concentrations on glycogen recovered from oyster tissue after a single precipitation*

(All samples were treated with 20 ml. of lye, diluted with 100 ml. of water and 125 ml. of absolute alcohol)

SAMPLE NO.	NaOH	TIME	SAMPLE WEIGHT	GLYCOGEN
	<i>per cent</i>	<i>hours</i>	<i>grams</i>	<i>per cent</i>
59	50	1	6.29	2.68
60	50	1	6.10	2.58
61	30	1	5.98	2.48
62	30	1	6.03	2.57
63	30	$\frac{1}{2}$	6.03	2.54
64	30	$\frac{1}{2}$	6.46	2.61
67	30	$\frac{1}{2}$	5.99	2.62
68	30	$\frac{1}{2}$	6.01	2.54

30 per cent, and a second precipitation are equally as needless as has been shown to be the case for glycogen determinations in muscle and liver (4).

In Table 8 the weight of tissue used was reduced to approximately 6 grams and the volume of lye to 20 ml., and a single precipitation was used throughout. Here again the 30 per cent lye and 15 minute digestion gave results in excellent agreement with those obtained by the use of a stronger lye for a longer period.

In order to test the milder digestion conditions still further one lot of especially "fat" oysters† was analyzed. By using samples approximating both 6 and 10 grams the experiments were made to cover all conditions

TABLE 9.—*Effect of digestion at 80°C. with varied time intervals and quantities of lye on the glycogen recovered from oyster tissue with a single precipitation*

SAMPLE NO.	NaOH		TIME	PPTS	SAMPLE WEIGHT	GLYCOGEN
	<i>per cent</i>	<i>volume</i>	<i>hours</i>		<i>grams</i>	<i>per cent</i>
71	50	25	1	1	10.39	6.12
72	50	20	1	1	6.35	6.13
73	30	25	$\frac{1}{2}$	1	10.01	6.19
74	30	20	$\frac{1}{2}$	1	6.59	6.11
75	30	25	1	1	10.17	6.11
76	30	20	1	1	6.52	6.24
77	30	25	$\frac{1}{2}$	1	9.96	6.06
78	30	20	$\frac{1}{2}$	1	5.83	6.14

Average for the 6 gram samples, 6.15

Average for the 10 gram samples, 6.12

All odd samples were diluted with 125 ml. of water and 150 ml. of alcohol, while even samples were diluted with 100 ml. of water and 125 ml. of alcohol.

† The writers wish to express their thanks to Graham Evans of the Chesapeake Corporation, West Point, Virginia, for these animals, which he collected from beds on the bottom of Queens Creek in the lower part of the York River in Virginia.

likely to be encountered in the course of determining glycogen in oyster tissue. A study of the results (Table 9) shows that, although the glycogen values obtained by the digestions with 30 per cent lye show a greater variation than do the two digestions with 50 per cent lye, all are within the limits of error for the method.

Since other investigators (7) had shown that heating the alkaline alcoholic solution to boiling and cooling to room temperature effect a complete precipitation of glycogen from mammalian tissue, two experiments were made along similar lines with oyster tissue, but these yielded questionable results.

For the present it is recommended that the glycogen precipitates stand overnight before filtration.

The results show that digestion of ground oyster tissue at a lye temperature of 80° C. for 30 minutes with 2 ml. of 30 per cent sodium hydroxide per gram of tissue is ample to secure complete liberation of all the glycogen present. Dilution of the alkaline hydrolyzate with 5 volumes of water and the addition of 6 volumes of 95 per cent alcohol give complete precipitation of the glycogen in a condition of sufficient purity to make reprecipitation unnecessary. The glycogen is thus obtained in a physical condition that permits, with the proper grade of filter paper, rapid filtration and washing of the precipitate. The glycogen isolated by a single precipitation is leached from the filter with hot water as readily as that subjected to two precipitations.

Occasionally there was encountered a precipitate prepared by the recommended procedure which, on the addition of hot water, leached from the filter much more slowly than the others. No detail of manipulation could be found responsible for this, but a careful survey of all cases led to the conclusion that it is caused by the actual quantity of glycogen on the filter; with a fixed quantity of hot water the large precipitates require a disproportionately longer time for peptization and hence their suspensions pass through the filter much more slowly than the suspensions from the smaller precipitates. Therefore it is suggested that the quantity of ground tissue taken for digestion be so selected as to yield not over 0.4 gram of precipitated glycogen, and that to avoid sampling errors the entire suspension of the latter be hydrolyzed. For the dextrose determination there should be chosen an aliquot part of this hydrolyzed solution that will contain a quantity of glucose lying within the most desirable portion of whatever empirical dextrose table is being used (8).

#### GLYCOGEN, ITS HYDROLYSIS AND DETERMINATION

All quantitative determinations of glycogen were made by hydrolysis of its aqueous suspension with hydrochloric acid, and estimations of the dextrose present were made in an aliquot of the resulting solution by use of the Hagedorn-Jensen procedure. Since the problem underlying this

work did not require an accuracy greater than 0.1 per cent the great convenience of the Hagedorn-Jensen method, with its iodometric titration and complete absence of precipitations, was considered to outweigh, by far, the error introduced by the large dilution factor (2500:1) required. Early in the course of the quantitative determinations it was found that the acid concentration, length of the heating period, and temperature of heating as specified by different workers (9) for the complete conversion of glycogen to glucose not only varied widely, but also constituted a large source of errors in the determination. In order to obtain concordant results in the hydrolysis of glycogen to dextrose considerable experimental work was necessary upon the acid concentration, length of the heating period, and the heating temperature. Only the procedure as finally used is given in this paper, and the detailed experimental work by which this was established will be published elsewhere.

#### RECOMMENDED PROCEDURE

##### DIGESTION OF TISSUE

Weigh by difference into a 400 ml. beaker a quantity of the finely ground wet oyster tissue (5-10 grams) that will yield a final precipitate of not more than 0.4 gram of glycogen. Add to the sample 10-20 ml. (2 ml. per gram of tissue) of 30 per cent carbonate-free NaOH (3+7), cover the beaker with a watch-glass, and digest on the steam bath at  $80^{\circ}\text{C.} \pm 10^{\circ}$  (temperature of digesting mixture) with occasional stirring for 30 minutes.

##### PRECIPITATION AND ISOLATION OF THE GLYCOGEN

Remove the beaker from the bath, disintegrate any lumps with a mushroomed stirring rod, add 100 ml. of water, mix thoroughly, and add 135 ml. of 95% alcohol. Stir only enough to secure a uniform solution. Cover the beaker and set aside at room temperature for 10-12 hours or overnight to allow the precipitated glycogen to settle. Decant the supernatant liquid through a 18.5 cm. Whatman No. 54 filter folded at  $90^{\circ}$ , using a fluted funnel. Wash the precipitate four times by decantation with 75 ml. portions of 66% alcohol (2 volumes of 95% alcohol + 1 of water), then transfer the precipitate to the filter and wash twice with 66% alcohol. (If desired, the filters may be covered with watch-glasses, but when the tissue is digested in the manner recommended filtration is so rapid that the danger of the precipitate drying on the filter is remote. If the oysters were free from mud the precipitate is usually white before the washing by decantation is completed. With muddy oysters, however, no amount of washing with alcohol will remove the grey color from the precipitate.)

##### SOLUTION AND DETERMINATION OF THE GLYCOGEN

After the washing is completed, close the bottom of the funnel by a piece of rubber tubing and a pinch-clamp. Fill the filter with hot water, cover with the watch-glass, and let stand 2-3 hours or overnight. Open the pinch-clamp and allow all the solution to drain from the filter into a 250 ml. volumetric flask. Close the funnel with the pinch-clamp and fill with hot water as before. Allow this water to remain in the funnel at least one hour, then drain as before. At first the glycogen solution is quite turbid. Continue the washing with hot water until the filtrate is quite clear, but keep the total volume of filtrate and washings within 175 ml.

To the aqueous solution of the glycogen add 12.5 ml. of concentrated HCl (sp. gr.

not less than 1.185), mix, and immerse the flask in a boiling water bath (temperature of solution in the flask must not be less than 92°) for at least 4 hours.‡ Cool the mixture, neutralize to phenolphthalein indicator with 20% NaOH, cool again, dilute to volume with water, and determine the dextrose in an aliquot of this solution by any of the accepted methods. The corresponding weight of dextrose  $\times 0.9$  equals its equivalent of glycogen. Correct this result for dilution to obtain the percentage of glycogen in the sample.

### SUMMARY

(1) The work described in this paper was limited to the glycogen from oyster tissue. On the basis of statements in the literature, however, it is believed that the recommended procedure would work equally as well on tissues from other animals.

(2) The recommended procedure for the isolation of glycogen has the following advantages over the method now used in official work:

- (a) A more dilute lye is used.
- (b) Relatively cheap sodium hydroxide replaces the expensive potassium compound.
- (c) The time required for disintegration of the tissue is reduced to one-fourth of that now specified.
- (d) The temperature specified for the disintegration of the tissue is probably lower than that intended by the present A.O.A.C. method.
- (e) The milder temperature specified for disintegration of the tissue liberates the glycogen in a physical condition that is much better suited to the subsequent operations.
- (f) The improved physical condition of the isolated glycogen enables the analyst to complete the determination, not only with much less effort, but also within 40 per cent of the time required by the procedure now in use.
- (g) The filter paper used for collection of the precipitated glycogen is an important factor in this great saving of time and effort.
- (h) The glycogen is obtained in such a high state of purity that only a single precipitation is necessary.
- (i) The quantity of alcohol needed for a determination has been reduced to 35 per cent of that required by the present procedure.

(3) Complete hydrolysis of the glycogen could not be obtained in three hours at 98° C. when the acid concentration specified in the A.O.A.C. method was used.

(4) The use of 12.5 ml. of hydrochloric acid (sp. gr. not less than 1.185) is recommended for each 175 ml. of an aqueous solution of glycogen, and hydrolysis should be carried out at a temperature of not less than 92° for at least 4 hours.

Williamsburg, Virginia, June 17, 1940.

### REFERENCES

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‡ In work being reported elsewhere it was found that samples of glycogen gave the same glucose values even though hydrolysis was allowed to continue for 24 hours. It is thus possible to take advantage of the convenient practice of allowing the hydrolysis to proceed overnight.

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## DETERMINATION OF PEEL OIL IN GRAPEFRUIT JUICE\*

By W. C. SCOTT (U. S. Bureau of Agricultural Chemistry and Engineering,  
Fruit and Vegetable Products Laboratory, Weslaco, Texas)

The adoption by the juice canning industry of a number of types of mechanical juice extractors has shown the need for a method to measure the quantity of peel oil incorporated in the juice during extraction. The presence of small quantities of essential oil in fresh juice may improve its flavor (1), but under unfavorable storage conditions excessive oil in the canned product contributes to the development of a distinct turpentine-like flavor. This deterioration, which is probably due to the oxidation of the terpene fraction of peel oil (4), occurs in direct proportion to the quantity of oil present. Advantage may therefore be taken of the volatility of this fraction for its distillation and recovery, as the quantity of oil thus recovered would be as effective a measure of the undesirable constituents incorporated in the juice during extraction as would be the total amount of oil present.

Demonstration of the method presented for measuring recoverable oil content has already led to its adoption by grading agencies (5) as one of the tests to be applied to commercial packs of grapefruit juice. The method has also been used in this Laboratory for several seasons, and has been found to indicate satisfactorily the relative quantities of oil in juices extracted by various methods. Methods described in the literature for measuring essential oils in vegetable oils (2) and in ground citrus pulp (6) are not satisfactory owing to the very small quantities of oil recoverable from palatable juices.

## METHOD

In principle, the method is merely steam distillation and the recovery of

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\* Food Research Unit Contribution No. 447.



volatile oil in a receiver suitable for measuring a small quantity of oil lighter than water. The apparatus is shown in Figure 1.

The 3 liter flask A is used for the generation of steam, but it may be replaced by any more convenient source that supplies an easily controlled flow of clean steam to the sample in the 5 liter flask B. Samples less than

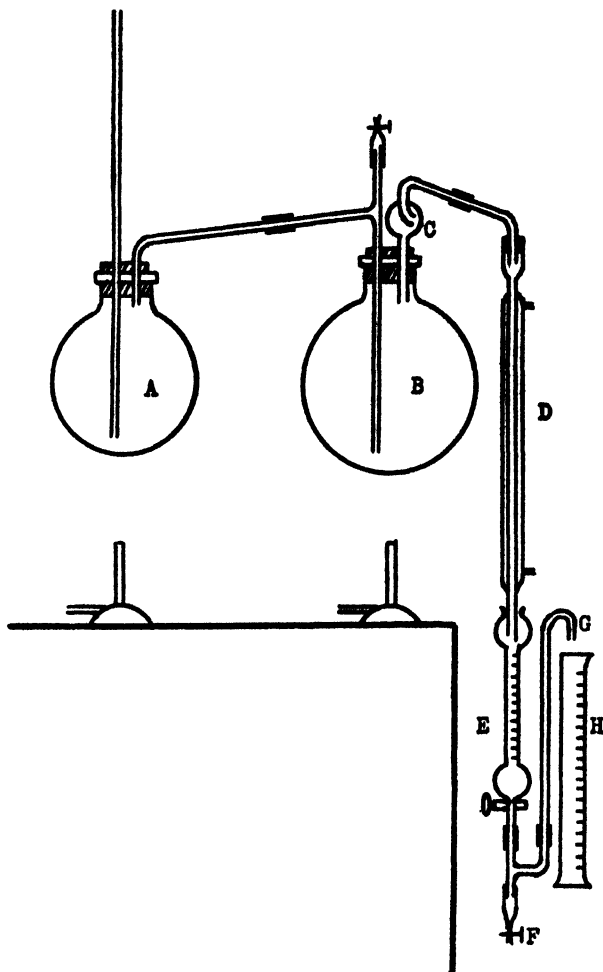


FIG. 1.—APPARATUS FOR THE DETERMINATION OF PEEL OIL IN CITRUS JUICE

3 liters in volume are not recommended, because the yield of oil from smaller samples is difficult to measure. Direct heat is applied to bring the sample to boiling as quickly as possible, then the distillation is finished by steam from the generator. Care must be taken to control the rate of distillation in order to prevent the escape of uncondensed vapor.

Use of a Kjeldahl connecting bulb C is recommended. When a single large

tube was substituted the oil emulsified in the receiver in practically every trial; even when the Kjeldahl bulb was used, emulsification occurred very frequently when the rate of distillation was rapid enough to yield 125 ml. of distillate in less than 15 minutes.

Use of the 18 inch condenser D in a vertical position, rather than the conventional 75° inclination, is recommended because oil condenses farther down the tube than does water, and unless washed down will cling to the tube and not be available for measurement. The tip of the condenser should barely clear the surface of the water in the receiver E. This level should be maintained high enough above the constricted portion of the receiver to prevent the main body of the condensed oil from entering the constricted part. If large drops of oil enter the small tube during distillation, it may be difficult to reunite them with the main body of oil.

Use of a Bromwell fusel-oil separatory funnel is recommended because the constricted portion, graduated to 0.02 ml., facilitates accurate measurement of the oil, and the lower bulb serves to collect any droplets that may have become separated from the main body of oil and been washed down through the small tube by the flow of condensed water. Upon completion of the distillation these droplets will rise and unite with the main body of oil for measurement.

Use of the constant-level overflow tube G is optional, but it eliminates the necessity for keeping continuous watch on the level of liquid in the receiver.

A Wilson receiving flask of the Florentine type (2) may be substituted for the Bromwell funnel and the overflow tube, provided the neck is graduated to allow reading to 0.02 ml. and the height of the siphon is adjustable. Such a flask is not listed in popular laboratory supply catalogs.

After distillation is completed, the stopcock F is turned to allow drainage until both surfaces of the floating oil are within the graduations of the narrow tube. Five minutes should be allowed for complete drainage of oil from the sides of the receiver and for the rise of droplets carried down to the lower bulb. The volume of oil may then be read in milliliters, and divided by the volume of the sample in milliliters, to get the percentage of recoverable oil by volume.

Tests indicate that prolonged distillation is accompanied by a small decrease in the yield of oil, due to its slight solubility in water. Measurement of oil recovered at various stages of the distillation indicated that additional oil was being collected up to 100 ml. of distillate, and that the change in volume of oil per additional 50 ml. of distillate was very small. These tests were made on samples to which 0.5, 1.0, 1.5, and 2.0 ml. of cold-pressed oil were added. It was estimated that 125 ml. gives the maximum yield of oil in the majority of cases, and therefore this amount was collected in the determinations reported.

## DISCUSSION

As indicated previously, this method does not measure the total quantity of peel oil originally present in the juice. Peel oil contains appreciable quantities (3, 4) of non-volatile wax as well as a large quantity of terpenes

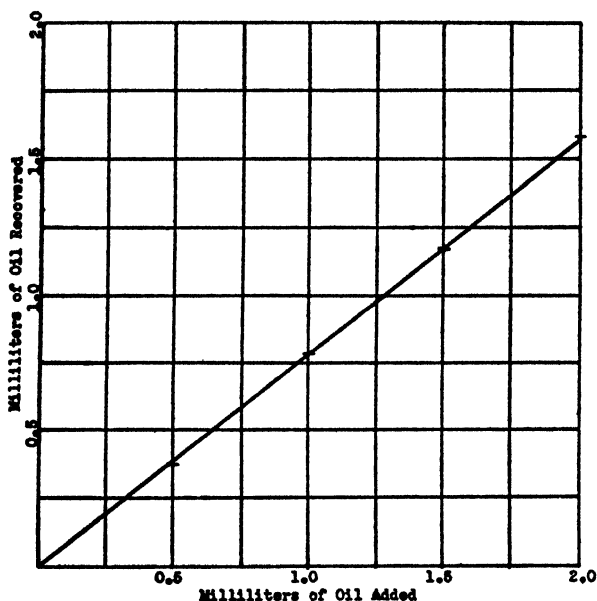


FIG. 2.—GRAPH SHOWING CONSTANT RELATION BETWEEN OIL ADDED (OR TOTAL OIL PRESENT) AND OIL RECOVERABLE

and aldehydes of widely varying volatility. Under the conditions of this determination, all the volatile constituents may not be recovered, but that those thus recovered do have a definite relation to those originally present in the juice was shown by adding measured quantities of cold-pressed peel oil to aliquot portions of carefully hand-reamed juice, and distilling. Results of this test are shown in Table 1 and Figure 2.

TABLE 1.—*Recovery of oil from hand-reamed juice*

OIL ADDED		OIL RECOVERED		PERCENTAGE RECOVERY
ml.	per cent	ml.	per cent	
0.00	0.000	Trace	0.001 ±	—
0.50	0.017	0.37	0.012	74
1.00	0.033	0.78	0.026	78
1.50	0.050	1.17	0.039	78
2.00	0.067	1.58	0.053	79
Average				77.25

Since the total oil content of two samples of juice under comparison would have no greater significance than that of the oil recoverable from them, it is not recommended that oil recoverable be converted into terms of total oil contained. This would be possible, however, by referring to Figure 2, and finding the quantity of oil added corresponding to the actual quantity of oil recovered, or by multiplying the oil recovered (ml.) by 1.3 (see Table 2:  $1/.7725 = 1.294$ ).

The quantity of oil recovered from commercially canned citrus juices is shown in Table 2, which summarizes the tests made in this laboratory during the 1938-39 season. The larger variations shown may have resulted from different adjustments of the mechanical extractors, but variations in conditions of the fruit, as maturity, peel thickness, and turgidity, have significant bearing on the quantity of oil released under any process. Distillation of aliquot portions of a thoroughly mixed sample show a maximum variation of 0.004 per cent at higher concentrations.

TABLE 2.—*Oil recovered from commercially canned citrus juices*

PRODUCT	METHOD OF EXTRACTION	TOTAL OIL RECOVERED
		<i>Per cent by volume</i>
Grapefruit Juice	Reamed by hand	Trace-0.005
Grapefruit Juice	Belt-type extractor used	0.016-0.017
Grapefruit Juice	Wheel-type extractor used	0.005-0.016
Grapefruit Juice	Perforated cylinder extractor used	0.050-0.086
Grapefruit Juice	Fruit grated and pressed	-0.075

Tests made during the 1939-40 season substantiated the practicability of the method presented, but as was expected, improvements in mechanical extractors led to considerably less peel-oil contamination than was normal for machines in 1938-39.

Although this method was devised for measuring the peel oil in grapefruit juice, it was applied in a number of instances to orange juice. Since the physical characteristics of orange oil are different from those of grapefruit oil, it would be expected that the percentage recovery of volatile constituents would be different. However, for the purpose of comparing the quantity of volatile oils in various lots of orange juice the method should be sufficiently accurate for commercial application.

#### SUMMARY

A method is presented for the measurement of peel oil in citrus juices by steam distillation and recovery of the volatile constituents. It is supported by data showing the constant relation between recoverable oil and the quantity originally incorporated in the juice. The range of oil recovered from juices extracted by various commercial processes in 1938-39 is also presented.

## ACKNOWLEDGMENT

The writer is indebted to J. L. Heid, chemist in charge of this laboratory; T. H. Baker, chemist for the Rio Grande Valley Citrus Exchange Juice Plant, at Mission; and to L. M. Emanuel, of the Fruit Testing Corporation, Orlando, Florida, for valuable suggestions concerning the refinement of this method.

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## DETERMINATION OF TANNINS BY PHOTOCOLORIMETER

By M. ROSENBLATT and J. V. PELUSO (Schenley Distillers Corporation, New York, New York)

In 1912, Folin and Denis (4) reported an investigation concerning phosphotungstic-phosphomolybdic compounds as color reagents. One of these compounds (designated hereafter as Folin-Denis reagent), was found to give a positive reaction with all oxy-phenyl compounds and, in particular, with tannic acid and pyrogallol. The presence of more than one oxy-phenyl bond in tannins makes the application of this reaction distinctly suitable for these compounds. Folin and Denis reported that the method was sensitive to 1 p.p.m. and that aliphatic compounds did not interfere. In another paper (5), they reported that the maximum color is developed within 10 minutes.

✓In 1921, R. D. Scott (6) utilized this reaction for the determination of phenols in water and recommended the use of 1-2 ml. of the Folin-Denis reagent. In 1922, Scott (7) further applied the reaction to tannins in whiskey, recommending 1 ml. of the Folin-Denis reagent and accepting the 10 minute time interval for optimum color development.

The colorimetric method, as applied to tannins in wines and whiskey, possesses distinct advantages over the official volumetric method (2). The latter involves, in addition to a tedious preparation of boneblack, a double oxidation-reduction titration and boneblack absorption, neither of which is specific for tannin. Indeed, the official method determines both tannin and color combined. But any compound capable of reducing potassium permanganate and of being absorbed by boneblack would materially alter the result. It is to be noted that a great variety of substances possess these two properties (1), and among these are the higher alcohols normally

present in alcoholic beverages. An absorbent for tannin has been persistently sought in order to make this method specific for this substance, but the complexity and variety of tannins present in nature have made any attempt unsuccessful (1). The colorimetric method, on the other hand, is more specific since only the restricted class of compounds containing the oxy-phenyl bond will affect the result. In addition, the photo-colorimetric method is clearly simpler and less liable to errors of technic and observation.

The work outlined here offers a means of defining the optimum standard conditions for the colorimetric method that will yield a more precise determination than has heretofore been available.

#### APPARATUS AND MATERIALS

The analysis of the blue color, produced by the reaction, was determined by a Hilger medium quartz spectrograph equipped with a Spekker photometer.

The measurements were made with a null-type photometer having two photoelectric cells (rectifier type) (9) balanced by external resistance through a galvanometer. The resistance dial read directly in units of per cent transmission. The source of light was a 100 W. tungsten filament lamp. One each of a matched pair of Jena UG2 1 mm. glass filters was placed before each photoelectric cell. Circular windows in a Bakelite absorption cell provided a liquid thickness of 2.5 cm.

The tannic acid standard was prepared from reagent-grade tannic acid by dissolving 0.100 gram in water and making up to 1 liter at 25° C. Correction was made for the moisture present in the solid tannic acid.

The Folin-Denis reagent was prepared by adding 100 grams of sodium tungstate, 20 grams of phosphomolybdic acid, and 50 ml. of 85 per cent phosphoric acid to 750 ml. of distilled water. The mixture was refluxed for 2 hours, cooled to 25° C., and diluted to 1 liter.

The saturated sodium carbonate solution used was obtained from a supersaturated solution that was seeded at 25° C. several hours before use.

Precautions were taken to have the reagents nitrate-free.

#### REACTION OF FOLIN-DENIS REAGENT WITH TANNIC ACID

Standards were prepared by adding 0.50–5.0 ml. of the standard tannic acid solution to 100 ml. volumetric flasks containing approximately 75 ml. of distilled water. The Folin-Denis reagent (0.5–5 ml.) was added, then the saturated sodium carbonate solution (1–10 ml.), and the solution was made to mark immediately. A blank containing the reagents without tannic acid was run with each set for the 100 per cent transmission determination. The color formed was examined photometrically from 2 minutes to 24 hours after the volume was made to mark. Except in the study of the temperature effect, the solutions were maintained at 25°

$\pm 0.5^\circ$  C. for color development. The solutions were stored in the dark and kept sealed during tests except when readings were being taken.

Since this determination may also be conducted for visual color comparison in Nessler tubes, the effect of light (direct sunlight) and of exposure to air was also investigated.

**Spectral Transmission of Blue Color.**—The characteristic spectral transmission curves for different concentrations of tannic acid after 1 hour's reaction with Folin-Denis reagent are shown in Figure 1. It is apparent

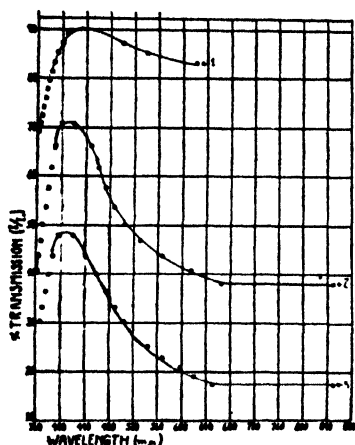


FIG. 1.—SPECTRAL TRANSMISSION OF THE COLOR FORMED BY THE FOLIN-DENIS REAGENT—TANNIC ACID REACTION

Folin-Denis reagent, 2 ml. per 100 ml.; Sat'd  $\text{Na}_2\text{CO}_3$ , 5 ml. per 100 ml.; temp.,  $25^\circ\text{C}$ .; time, 1 hour.

Concentration of Tannic Acid (mg. per 100 ml.): (1) 0.0, (2) 0.1, (3) 0.2.

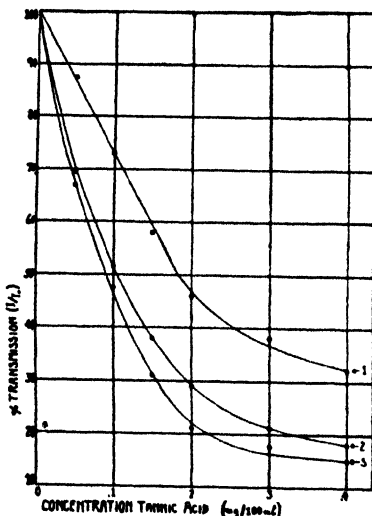


FIG. 2.—EFFECT OF WAVELENGTH ON THE RELATIONSHIP BETWEEN TRANSMISSION AND TANNIC ACID CONCENTRATION

Folin-Denis reagent, 2 ml. per 100 ml.; Sat'd  $\text{Na}_2\text{CO}_3$ , 5 ml. per 100 ml.; temp.,  $25^\circ\text{C}$ .; time, 1 hour.

(1) 450  $\text{m}\mu$ , (2) 550  $\text{m}\mu$ , (3) 650  $\text{m}\mu$ .

that there is no sharp minimum transmission, and the choice of a filter is therefore not limited to any narrow wave-length region in order to obtain maximum sensitivity. Any filter transmitting a narrow wave band that lies within the broad range covered by the minimum plateau (640–850  $\text{m}\mu$ ) will be satisfactory.

Derivative curves from Figure 1 were constructed by plotting the transmission against the tannic acid concentration for various wave lengths and are shown in Figure 2. The values were corrected for the transmission of the blank. It is clear that the maximum sensitivity of the determination will be obtained when the rate of change of transmission with

tannic acid concentration is a maximum. Figure 2 indicates that this condition is attained with wave lengths of 650  $m\mu$  to at least 850  $m\mu$ .

The characteristics of the colorimeter are such as to yield a high photoelectric response within the region 525–750  $m\mu$ . Although the sensitivity of the rectifier type of photocell is low in the red region (650  $m\mu$ ), this factor has little effect when the cell is used with a tungsten incandescent lamp where the spectral energy distribution is considerable, from 650 to 900  $m\mu$ .

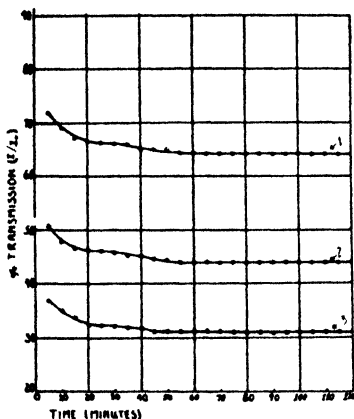


FIG. 3.—EFFECT OF TIME ON DEVELOPMENT OF COLOR FORMED BY FOLIN-DENIS REAGENT WITH TANNIC ACID

Folin-Denis reagent, 2 ml. per 100 ml.; Sat'd  $\text{Na}_2\text{CO}_3$ , 5 ml. per 100 ml.; temp., 25° C.; filter, UG2.

Concentration of Tannic Acid (mg. per 100 ml.): (1) 0.1, (2) 0.2, (3) 0.3.

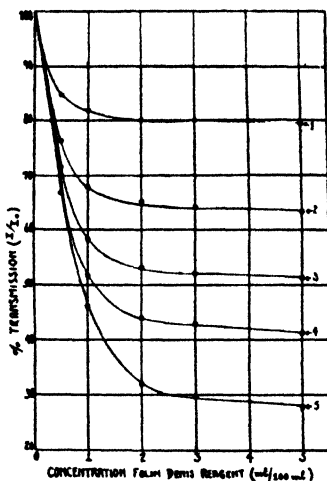


FIG. 4.—EFFECT OF FOLIN-DENIS REAGENT CONCENTRATION ON COLOR REACTION WITH TANNIC ACID

Sat'd  $\text{Na}_2\text{CO}_3$ , 5 ml. per 100 ml.; temp., 25° C.; filter, UG2; time, 1 hour.

Concentration of Tannic Acid (mg. per 100 ml.): (1) 0.05, (2) 0.10, (3) 0.15, (4) 0.20, (5) 0.30.

The filter chosen for this work was a Jena UG2, 1 mm. (3), which exhibits a suitable transmission band in the red region, having a maximum at 770  $m\mu$ . The violet and ultraviolet characteristics of this filter are of minor consequence since the tungsten filament lamp emits negligible energy in this region. The writers have since located a compound filter, KS62, 10 mm. (8), with greater transmission in the red, having a narrow range from 620 to 680  $m\mu$  and a maximum transmission at 650  $m\mu$ . This filter is highly recommended for high sensitivity in this determination.

*Effect of Time on Stability of Color.*—The color deepens rapidly during the first 15–20 minutes, and more slowly during the next 40 minutes



(Figure 3). At the end of an hour, the intensity of the color is stabilized and remains so for at least another hour. These data were obtained by using 2 ml. of the Folin-Denis reagent and 5 ml. of the saturated sodium carbonate solution per 100 ml.

It is therefore necessary that the color readings be made at least 1 hour after the reaction has started and, at most, after 2 hours. The 10 minute time period recommended by Folin and Denis and accepted by Scott is clearly unsatisfactory since, at this point, the transmission is changing rapidly with time. For all the work reported here, readings were taken 1 hour after mixing.

*Effect of Folin-Denis Reagent Concentration.*—The effect of Folin-Denis reagent concentration on the intensity of color development is shown in Figure 4. The data are based upon photometric observations, 5 ml. of saturated sodium carbonate per 100 ml. and 1 hour time interval prior to color reading being used. The intensity increases rapidly with increasing concentration of Folin-Denis reagent up to 2 ml. per 100 ml. and then increases at a very slow rate, especially at lower concentrations of tannic acid. It can also be seen that for concentrations of Folin-Denis reagent greater than 2 ml. per 100 ml., the effect of change in the Folin-Denis concentration upon transmission is practically zero.

Derivative curves were constructed from Figure 4 by plotting the negative log of the transmission (density) against concentration of tannic acid for various Folin-Denis reagent concentrations (Figure 5). These curves clearly show that at concentrations of Folin-Denis reagent less than 2 ml. per 100 ml., not only is the slope very small (indicating low sensitivity), but Beer's law is invalid throughout the complete range of tannic acid concentration. For concentrations of Folin-Denis reagent of 2 ml. per 100 ml. and higher, the curves are almost colinear (obeying Beer's law for a greater portion of the curve), and there is no advantage to be gained in using higher concentration of the reagent. For all the work reported here, solutions were made up with 2 ml. of Folin-Denis reagent per 100 ml.

*Effect of Sodium Carbonate Concentration.*—The effect of sodium carbonate concentration on the intensity of color development is shown in Figure 6. The data are based on photometric observations when 2 ml. of Folin-Denis reagent per 100 ml. and 1 hour time interval prior to reading were used. The intensity increases rapidly with increasing concentration of sodium carbonate up to approximately 4 ml. of saturated sodium carbonate solution per 100 ml. A broad minimum transmission is observable from 4–6 ml. at lower concentrations of tannic acid and 5–7 ml. at higher concentrations of tannic acid. At higher concentrations of sodium carbonate (above 8 ml. per 100 ml.) fading begins. It is clear, therefore, that 5–6 ml. of the sodium carbonate solution would be satisfactory from the standpoint of maximum color development and minimum change in transmission with changes in sodium carbonate concentration.

Derivative curves were constructed from Figure 6 by plotting the density against concentration of tannic acid for various sodium carbonate concentrations (Figure 7). It is readily seen that the maximum slope is obtained at 3 ml. of saturated sodium carbonate solution or higher. The proximity of the lines and the identity of slope in the range of 3–9 ml. indicate that from a consideration of sensitivity any of these sodium carbonate volumes would yield satisfactory results. However, the additional considerations obtained from Figure 6 narrow the range to 5–6

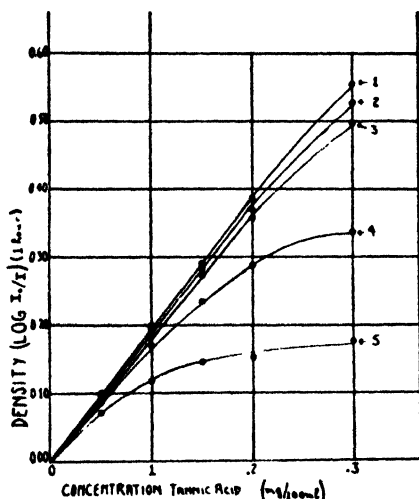


FIG. 5.—RELATION OF TANNIC ACID CONCENTRATION TO DENSITY FOR VARYING FOLIN-DENIS REAGENT CONCENTRATIONS

Sat'd  $\text{Na}_2\text{CO}_3$ , 5 ml. per 100 ml.; temp., 25° C.; filter, UG2; time 1 hour.

Concentration of Folin-Denis Reagent (ml. per 100 ml.): (1) 5.0, (2) 3.0, (3) 2.0, (4) 1.0, (5) 0.5.

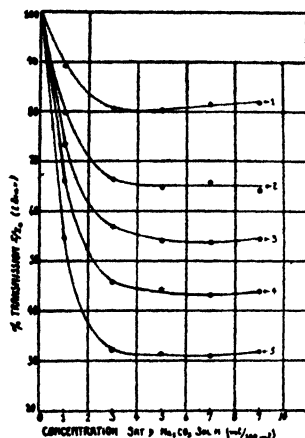


FIG. 6.—EFFECT OF SODIUM CARBONATE CONCENTRATION ON THE FOLIN-DENIS REAGENT—TANNIC ACID COLOR REACTION.

Folin-Denis Reagent, 2 ml. per 100 ml.; temp., 25° C.; filter, UG2; time, 1 hour.

Concentration of Tannic Acid (mg. per 100 ml.): (1) 0.05, (2) 0.10, (3) 0.15, (4) 0.20, (5) 0.30.

ml., and 5 ml. of the sodium carbonate solution was chosen as the minimum volume compatible with optimum color development and maximum stability.

*Effect of Temperature.*—The effect of temperature is appreciable (Figure 8). If the effect at 60 minutes, the time chosen for the standard procedure, is considered, it will be noted that at the lower concentration (0.1 gram per 100 ml.), the difference between 10° and 30° C. values is approximately 10 per cent of the mean value when transmission readings are converted to density. At the higher concentration (0.2 gram per 100 ml.), the difference for the same interval is again approximately 10 per cent of

TABLE 1.—*Temperature effect in terms of transmission*

TANNIC ACID CONCENTRATION	TRANSMISSION	TEMPERATURE EFFECT
mg./100 ml.	per cent	per cent transmission/°C.
0.05	79.2	0.1
0.10	64.1	0.1
0.15	52.2	0.15
0.20	43.2	0.2
0.25	36.5	0.3

the mean value when expressed as density. If it is assumed that the density variation with temperature is linear for the interval 10°–30° C., then the temperature effect may be approximately expressed as 0.5 per cent of the density value per degree centigrade. However, the density is a log function of the transmission and the temperature effect cannot be expressed simply in terms of transmission.

A calculation to obtain the temperature effect in terms of transmission is shown in Table 1. Since the precision obtainable with this colorimeter is 0.4 per cent transmission, it is clear that the temperature must be maintained constant within 1° C. in order to retain maximum precision.

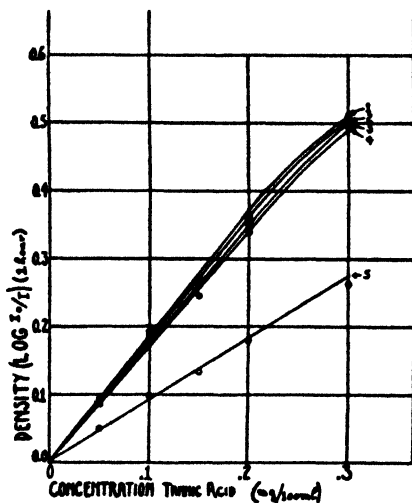


FIG. 7.—RELATION OF TANNIC ACID CONCENTRATION TO DENSITY FOR VARYING SODIUM CARBONATE CONCENTRATIONS

Folin-Denis Reagent, 2 ml. per 100 ml.; temp. 25° C.; filter, UG2; time, 1 hour.

Concentration of Saturated  $\text{Na}_2\text{CO}_3$  (ml. per 100 ml.): (1) 9.0, (2) 7.0, (3) 5.0, (4) 3.0, (5) 1.0.

The curves of Figure 8 also indicate that stability of color is attained more quickly at higher temperature. At 10° C., for both concentrations of tannic acid shown, the intensity is still increasing slowly at the end of 2 hours. At the higher temperature of 30° C., stability of color has been reached after 60 minutes, but 25° C. was chosen as the highest convenient temperature for normal laboratory purposes.

*Effect of Light.*—A set of tannic acid standards (0.0–0.5 mg. per 100 ml.) was divided into two equivalent sets and stored under different light conditions. Set A was allowed to remain in bright daylight for 6 hours, and Set B was stored in total darkness. The readings of Set A were indistinguishable from Set B when the precision of readings (0.4 per cent transmission) was considered. For the time needed for

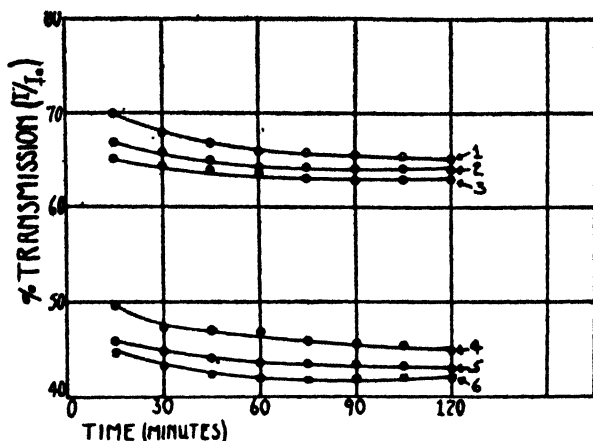


FIG. 8.—EFFECT OF TEMPERATURE ON THE FOLIN-DENIS REAGENT—TANNIC ACID COLOR REACTION

Folin-Denis Reagent, 2 ml. per 100 ml.; Sat'd  $\text{Na}_2\text{CO}_3$ , 5 ml.; filter, UG2; time, 1 hour.

(1), (2), and (3): 0.10 mg. Tannic Acid per 100 ml.

(4) (5), and (6): 0.20 mg. Tannic Acid per 100 ml.

Temp: (1) 10° C., (2) 22° C., (3) 30° C. (4) 10° C., (5) 22° C., (6) 30° C.

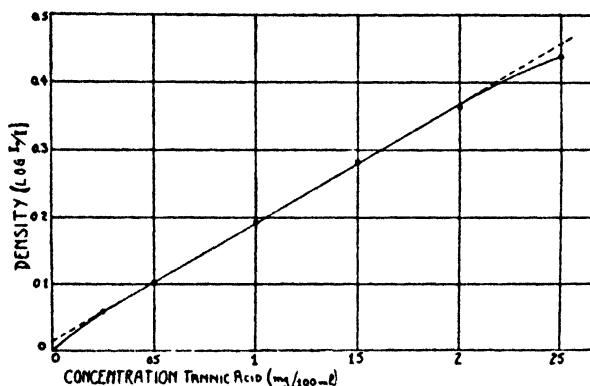


FIG. 9.—STANDARD CURVE: RELATION OF TANNIC ACID CONCENTRATION TO DENSITY

Folin-Denis Reagent, 2 ml.; Sat'd  $\text{Na}_2\text{CO}_3$ , 5 ml.; filter, UG2; time, 1 hour; temp., 25° C.

the test (1–2 hours), the effect of light is insignificant.

*Effect of Exposure to Air.*—A set of tannic acid standards (0.0–0.5 mg. per 100 ml.) was divided into two equivalent sets and permitted to stand under the following conditions: Set A in glass-stoppered narrow cylinders; Set B in open 250 ml. beakers. The solutions were permitted to stand for 2½ hours, and photometric readings were taken. The readings of Set A

plotted identically with those of Set B. For the time needed for the test (1-2 hours), the effect of air exposure is insignificant.

*Effect of Tannic Acid Concentration.*—In previous work involving the blue color formed by the Folin-Denis reagent with tannic acid, no attempt

TABLE 2.—Results obtained on known tannic acid solutions

TANNIC ACID CONCENTRATION	TRANSMISSION	DENSITY	MEAN DENSITY	AVERAGE DEVIATION OF MEAN
mg./100 ml.	per cent			per cent
0	100	0	0	—
.025	87.0	0.0607	.0592	1.3
	87.2	.0596		
	87.3	.0588		
	87.4	.0577		
.05	79.1	.1021	.1017	0.6
	79.0	.1021		
	79.4	.1004		
	79.1	.1021		
.10	64.0	.1937	.1933	0.4
	63.9	.1945		
	64.1	.1931		
	64.3	.1917		
.15	52.3	.281	.2822	0.2
	52.2	.282		
	52.1	.283		
	52.2	.282		
.20	43.1	.366	.3644	0.4
	43.4	.362		
	43.1	.366		
	43.2	.364		
.25	36.4	.439	.4377	0.3
	36.5	.438		
	36.4	.439		
	36.7	.435		

has been made to determine the region where Beer's law is valid. Under the standard conditions of test that have been chosen and described in detail immediately below, Beer's law is valid for tannic acid concentrations of 0.05-0.25 mg. per 100 ml. (Figure 9). At concentrations of tannic acid lower than 0.05 mg. and higher than 0.25 mg. deviations appear. This range was determined with filter UG2; preliminary results with filter KS64, offering a much narrower transmission band, indicate that the

range of validity of Beer's law may be extended to at least 0.35 mg. of tannic acid per 100 ml.

### PROCEDURE

A calibration curve (Figure 9) was constructed in the following manner: 0.5, 1.0, 1.5, 2.0, and 2.5 ml. of standard tannic acid solution (100 mg. tannic acid per liter) were pipetted into each of five 100 ml. glass-stoppered volumetric flasks containing approximately 75 ml. of distilled water; 2 ml. of the Folin-Denis reagent, then 5 ml. of the saturated sodium carbon-

TABLE 3.—*Results obtained on whiskey and wine*

SAMPLE NO.	VOLUME OF SAMPLE	TANNIC ACID ADDED	TRANS- MISSION	DENSITY	OBSERVED TANNIN CONTENT AS TANNIC ACID	RECOVERY OF ADDED TANNIC ACID	MEAN TANNIN CONTENT	
							OFFICIAL A.O.A.C.	PHOTO- METRIC
1 Whiskey	ml.	mg.	per cent		mg.	mg.	mg./ml.	mg./ml.
	0.250	—	52.9	0.277	0.150	—	0.63	0.601
	0.125	—	71.4	0.146	0.075	—		
	0.125	—	71.2	0.150	0.076	—		
	0.125	0.025	64.0	0.194	0.100	0.025		
	0.125	0.050	58.5	0.230	0.123	0.048		
2 Whiskey	1.00	—	39.9	0.399	0.22*	—	0.22	0.227
	0.50	—	60.4	0.219	0.116	—		
	0.50	—	60.9	0.215	0.114	—		
	0.50	0.025	54.3	0.265	0.143	0.028		
	0.25	0.050	61.7	0.210	0.112	0.055†		
3 Whiskey	0.250	—	46.2	0.335	0.182	—	0.65	0.727
	0.250	—	46.0	0.337	0.183	—		
	0.125	—	67.1	0.173	0.090	—		
	0.125	0.035	57.8	0.238	0.127	0.037		
	0.125	0.050	54.4	0.264	0.143	0.053		

\* At the end of region where Beer's law is valid.

† Recovery calculated from 0.50 ml. sample.

ate solution were added. The flasks were filled to mark with water, shaken well, and allowed to stand for 1 hour at 25° C. At the same time and along with the standards a blank was run with all the reagents in the absence of tannic acid. This blank yielded the corrected zero value for the calibration curve when the photometer was adjusted to read 100 per cent transmission with the blank in the cell. At the end of the hour, photometric readings were taken with the appropriate red filter (Jena UG2 or KS64). The transmission readings were transformed into density values (density =  $\log 1/T$ ) and plotted against concentration of tannic acid (Figure 9).

For unknown samples, a volume of sample that contained 0.05–0.25 mg. of tannins was pipetted into 100 ml. glass-stoppered volumetric flasks,

and the procedure was continued exactly as directed above, including a blank for the 100 per cent transmission setting. The transmission readings were transformed into density values, and the tannic acid contents were read directly from the calibration graph.

#### ACCURACY

A series of known tannic acid solutions was prepared in quadruplicate by the standard procedure, and individual determinations were made on each sample. The results are shown in Table 2.

The photometric measurements can be duplicated to within 0.4 per cent transmission, which calculates to about 0.5 per cent average deviation from the mean density within the region of validity for Beer's law (0.05–0.25 mg. tannic acid per 100 ml.). Since the density is linear with concentration of tannic acid for this interval, the accuracy of the method is 0.5 per cent provided the test conditions are exactly maintained.

Samples of whiskey and wine, with and without tannic acid, were examined photometrically for tannic acid content, and the results are shown in Table 3. The agreement among the samples without added tannic acid is excellent. The recovery of tannic acid is within 0.003 mg. for values of 0.025–0.050 mg. of added tannic acid.

It is to be noted that the tannic content of a sample is expressed in terms of tannic acid. Since the tannins are compounds of variable constitution, it is impossible to express results in terms of actual tannic content. Any phenolic compound may be used as a standard in this determination, but tannic acid was chosen because of its close structural relationship to the natural tannins.

#### SUMMARY

A spectral analysis of the blue color formed by the Folin-Denis reagent with tannic acid has been determined.

The variables affecting the development of the blue color and a standard procedure that gives maximum transmission and stability compatible with good sensitivity have been investigated.

The method is accurate to within 0.5 per cent.

#### ACKNOWLEDGMENT

The writers are grateful to A. J. Liebmann, Director of the Schenley Laboratories, for valuable suggestions and criticisms and are also indebted to E. Stevens and A. Wattenberg of the Spectrographic Division for the data on the spectral analyses and suggestions concerning the choice of a filter.

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## SEMIMICRO METHOD FOR DETERMINING COPPER REDUCED BY SUGARS\*

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In most of the micro and semimicro methods, the cuprous oxide formed by the action of sugars on alkaline copper solutions is determined without separating it from the solution in which it was formed.

The error caused by this procedure in the case of some plant extracts was noted in a previous paper (1), and a method for separating the cuprous oxide at the centrifuge was proposed. When standardized and used under exactly uniform conditions, this method has given excellent results, but it has been found that it does not recover quite all the cuprous oxide. It is possible that the slight loss may be due to reoxidation during centrifuging.

In an attempt to develop a better method the Bertrand titration (2) was studied. As it is usually described, this method is not applicable to small quantities of cuprous oxide, but excellent results have been obtained by modifying it, largely according to suggestions cited by Kolthoff (3).

The oxidizing solution is essentially Shaffer and Somogyi's No. 50 (4), but made in two parts and without potassium iodide.

The details of the modified method follow:

### REAGENTS

(a) *Oxidizing solution*.—(1) Dissolve 20 grams of  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  in water and dilute to 1 liter. (2) Dissolve 50 grams of Rochelle salts, 50 grams of  $\text{Na}_2\text{CO}_3$  (anhydrous), and 40 grams of  $\text{NaHCO}_3$  in water and dilute to 1 liter.

(b) *Ferric alum crystals*.—Dissolve 100 grams in water and dilute to 1 liter.

(c) *Sulfuric acid*.—Approximately 1+1.

(d) *Orthophosphoric acid*.—Approximately 25%.

(e) *Potassium permanganate*.—0.01 N.

(f) *Orthophenanthroline ferrous sulfate complex*.—0.025 M.

### DETERMINATION

To the sample in a final volume of 10 ml. in a large test tube (a 50 ml. centrifuge tube is convenient) add 5 ml. each of the two parts of the oxidizing solution. Set the tube in a boiling water bath and cover (a small inverted beaker may be used). After heating 15 minutes filter the reaction mixture through a König A2 filter (or other

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sintered-bottom filter of similar porosity) and wash with water. Change the receiver and dissolve the cuprous oxide with 10 ml. of the neutral ferric alum solution, used first in the tube, then in the crucible, where some stirring is necessary if fairly large quantities of the oxide are present. Smaller quantities are dissolved as the ferric alum solution is drawn through the bottom of the crucible. Wash the tube and crucible with 3 ml. of the  $\text{H}_2\text{SO}_4$  and with water.

To the receiver add 3 ml. of the  $\text{H}_3\text{PO}_4$  and one drop of the orthophenanthroline ferrous sulfate complex, and titrate the reduced iron with the  $\text{KMnO}_4$ . Run blanks on the reagents.

Equations showing the relation between mg. of sugar and ml. of 0.01 *N*  $\text{KMnO}_4$  (net titration) are—

For glucose,  $y = 0.018 + 0.212x$ ;

for fructose,  $y = 0.026 + 0.221x$ ; and

for sucrose,  $y = 0.022 + 0.205x$ .

In each equation  $y$  = mg. of the sugar and  $x$  = ml. of the  $\text{KMnO}_4$ .

The equations for glucose and sucrose cover the range from 0.05 to 4.0 mg. of the sugar. For fructose 0.05 mg. = 0.2 ml.  $\text{KMnO}_4$ . The equation applies to the rest of the range to 4.0 mg.

Bureau of Standards glucose and sucrose and Eastman Kodak Co. levulose were used for the standardization. They were dried in vacuo at 65° C. The sucrose was hydrolyzed by invertase.

Exact agreement with Shaffer and Somogyi's results is not to be expected, as their reagent contained a small quantity of potassium iodide and also slightly less cupric sulfate than the one used in this work. The comparison is given in Table 1, where the results are expressed as ml. of 0.01 *N* (calculated from Shaffer and Somogyi's results).

TABLE 1

SUGAR	GLUCOSE		FRUCTOSE	
	S & S	P	S & S	P
mg.				
2.50	11.40	11.70	—	—
2.00	9.18	9.35	8.95	8.93
1.00	4.55	4.63	4.40	4.40
.50	2.22	2.27	2.12	2.15
.25	1.08	1.10		

The method has been used satisfactorily with extracts of tomato leaves and of various parts of the timothy plant. It gives no additional blank with well washed yeast.

With suitable standardization, of course, the method for determining cuprous oxide could be used with other copper reagents.

#### SUMMARY

A modification of the Bertrand titration is described. It provides a convenient means for separating and determining small quantities of cuprous oxide formed by the action of sugars on alkaline copper reagents.

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LABORATORY STUDIES ON DEVELOPMENT  
OF MOLD IN CREAM

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The occurrence of mold, particularly *Oospora lactis*, in dairy products has been studied for a number of years. Of especial interest in recent years has been the study of the occurrence of *O. lactis* in cream intended for butter making. The writer (1, 2) has previously called attention to the fact that different lots of farm cream vary greatly in their content of vegetative mold, certain lots being generally free or nearly so of mold mycelia while others contain large quantities of mold. The present paper describes certain experimental work on the development of *O. lactis* in cream that helps to explain in part the differences in mold content found in commercial lots of cream.

The preliminary laboratory experiments were made with the object of finding what factors controlled the growth of *O. lactis*. Those briefly described here dealt with methods of air restriction, since this means had previously been found to retard the growth of mold in cream (3, 4). However, it soon became apparent that temperature and the relative quantities of mold inoculation were interrelated in their effect on mold development and apparently of more importance than air restriction alone.

## METHODS

The methods used, the methylene blue borax (MBB) test for mold in cream and the mold mycelia test for mold in butter, have been described (1, 2). In addition, the plating method with acidulated potato dextrose agar was used for the estimation of live molds.

*Preliminary Experiments on Control of Mold Growth*

*Air restriction.*—The restriction of air by means of tightly sealed jars, as studied by Parfitt and Galema (4), was tried in an early experiment, and their results were substantiated with respect to mold development.

Four clean, pint, Mason jars were filled three-fourths full with raw cream. The cream in two jars was inoculated with commercial lactic acid starter (25 ml. per jar), and the cream in the other two jars was left uninoculated. The top of one of each set of jars was then clamped down tightly, and the lids on the other two jars were left unsealed. All jars were

allowed to remain undisturbed at room temperature for 5 days, at which time visible mold was found to be present on the cream in the unsealed jars. No mold was visible on the cream in the sealed jars, and the MBB tests were negative. The addition of the starter did not alter the results significantly. An attempt was then made to extend the use of air restriction by layering the cream.

*Layering cream.*—One quart of fresh, raw, 40 per cent cream was added once a day for 6 days to each of two 2-gallon containers. Into one of these containers the cream was poured each day directly into the portion previously accumulated, and the whole mass was stirred thoroughly. In the other container the cream was added carefully each day in such a way as to overlay the cream already present. Two sets of conditions were thus provided, namely, layered and stirred. The containers were left at an air temperature of approximately 30°C. The procedure was then repeated, except that both containers were cooled to approximately 21°C. At the end of 6 days the lots were tested for mold, portions of each lot were churned after neutralization and pasteurization, and mold mycelia counts were made. The results obtained on the two trials are shown in Table 1.

TABLE 1.—*Effect of layering and stirring of cream on mold development*

TREATMENT	CONDITION OF CREAM	MOLD IN CREAM BY MBB TEST	MOLD MYCELIA COUNT IN BUTTER
		mm.	per cent
Layered, 21°C.	Smooth	0	0
Stirred,     "	"	0	4
Layered, 30°C.	Some layers gassy	0	2
Stirred,     "	Curdy	9	100

It will be noted (Table 1) that appreciable quantities of mold developed only in the samples that were stirred once a day and kept at air temperature. In order to check further the results obtained in the previous experiment, a similar test was made with smaller amounts of cream, a wider temperature range, and two additional methods of adding the cream.

Every day for 6 days 100 ml. of fresh cream from a well-mixed lot was added to each of twelve 600 ml. beakers. Four beakers were held at each of the following temperatures: 10°, 20°, and 30°C. The following methods of adding cream to the original 100 ml. in each beaker were used for each of the temperature conditions: 1. 50 ml. of fresh cream was stirred in, after which 50 ml. more was poured on the surface without stirring. 2. The whole addition was stirred into previous additions. 3. The fresh cream was layered. 4. The fresh cream was poured in with no attempt at stirring or layering. Each of the twelve lots was thus given a different treatment by a combination of the above factors. At the end of 7 days the samples were thoroughly mixed and tested for mold by the methylene blue borax

method. Butter was then churned from each sample, and mold mycelia counts were made. The results obtained are given in Table 2.

TABLE 2.—*Effect of temperature and method of treatment on mold development*

TREATMENT	TEMPERATURE	MOLD IN CREAM BY MBB TEST AT END OF 7 DAYS	MOLD MYCELIA COUNT IN BUTTER AT END OF 7 DAYS
	°C.	mm.	per cent
Stirred and layered	10	0	0
Stirred	10	0	0
Layered	10	0	0
Poured in only	10	0	0
Stirred and layered	20	0	0
Stirred	20	0	0
Layered	20	0	0
Poured in only	20	0	0
Stirred and layered	30	0	0
Stirred	30	6	95
Layered	30	0	0
Poured in only	30	4	61

As will be noted in Table 2 mold development was not detected when the cream was stored at 10° and 20°C. regardless of the treatment. At 30°C. mold development was significant when the cream was not layered.

**Stirring.**—Since it was considered that stirring cream twice daily might have a retarding action on mold development, another experiment was made in which once-a-day stirring was compared with twice-a-day stirring. Sixteen quart Mason jars were filled half full with cream, and variations in treatment were made as follows:

**STIRRING:** Half of the lots was stirred once a day, and the other half was stirred twice a day at approximately 12-hour intervals.

**TEMPERATURE:** Half of the lots was kept in a warm atmosphere, while the other half was kept somewhat cooler. The actual temperature for the "warm" lots varied from 27 to 30°C., with a mode of 29 to 29.5° and for the "cooled" lots from 23.5 to 25.0°C., with 24.5°C. the mode.

**INOCULATION:** One-half of the lots was inoculated with 5 ml. of a heavy suspension of *O. lactis* culture, and the other half was not inoculated.

**CREAM:** Two different batches of cream were used, one fresh cream and the other week-old sour cream.

Each of the 16 samples was given a different treatment by a combination of the above factors. All the samples were tested for mold from the third day on by the MBB test. The results are shown in Table 3.

In the experiment just described stirring twice a day retarded the growth of mold in the case of the week-old cream when the cream was kept

TABLE 3.—*Effect of stirring and temperature on mold growth*  
(Results given as mm. of mold by MBB method)

TREATMENT	NO. OF TIMES STIRRED PER DAY	TEMP.	TIME AFTER INOCULATION (DAYS)				
			3	4	5	6	7
Fresh cream—not inoculated	Once	29.5	0	0	0	0	0
	"	24.5	0	0	0	0	0
	Twice	29.5	0	0	0	0	0
	"	24.5	0	0	0	0	0
Fresh cream— inoculated	Once	29.5	8	8.5	9.5	9.9	10.5
	"	24.5	7	7.4	8.8	10.0	10.5
	Twice	29.5	6	6.2	8.2	7.1	8.0
	"	24.5	3.5	4.7	6.0	6.0	9.0
Week-old cream— not inoculated	Once	29.5	7.0	7.0	8.5	9.4	9.5
	"	24.5	1.2	1.5	5.5	5.8	7.2
	Twice	29.5	0	0	1.5	2.2	5.5
	"	24.5	0	0	0	0	1.0
Week-old cream— inoculated	Once	29.5	5.5	8.2	9.8	10.8	8.7
	"	24.5	2.7	1.8	6.0	5.8	8.1
	Twice	29.5	1.0	1.8	2.0	2.4	3.8
	"	24.5	0	0	1.0	0	0

cool. However, in fresh cream that had been inoculated stirring and cooling were effective in retarding mold growth only in the first few days.

It is interesting to observe that in the case of the fresh cream mold developed only in those cases where it had been introduced. In the old cream it is apparent that an inoculation of mold had already occurred. As will be noted in these cases the effect of stirring was variable and not of great significance by itself in preventing mold growth.

#### *Preliminary Experiments on Effect of Amount of Inoculum*

The question of the relation of amount of mold inoculum to the resulting growth arose as a consequence of the results obtained in the preliminary experiments previously described. In an early experiment fresh 30 per cent and 40 per cent creams were heavily inoculated with an *O. lactis* suspension and compared with the same cream without inoculation. Fresh cream was added once a day and stirred in, and the cream was held at 20°C. Mold layers developed on the inoculated cream, accompanied by positive MBB tests, but no visible mold developed on the uninoculated series and the MBB tests were negative. In further experiments in which variable quantities of inoculum were added to fresh raw cream it was found that there was a fairly close relation between the amount of inoculum and the amount of growth resulting on thin layers of cream held for

24 hours at room temperature. In this work portions of a heavy growth of *O. lactis* mold on cream were removed, thoroughly mixed, and added to uninoculated cream in amounts ranging up to 5 per cent by weight. In another trial in which 48 hour periods were used gross amounts of inoculum (.05-5 per cent) gave relatively the same growth responses. Amounts of inoculum less than .05 per cent of mold layer, however, were correlated with the mold development obtained. This observation is similar to that noted by Linossier (5).

#### EFFECT OF AMOUNT OF MOLD INOCULUM AND TEMPERATURE

The various experiments described indicate a possible interrelation of the amount of inoculum and temperature. In order to throw more light on the combined effect of these factors other experiments were made in which fresh cream was added at 12 hour intervals and stirred into the stored cream.

Three series of tests were run and the same general plan was followed for each series. In the first series eight sterile, quart, Mason jars were used, four of which were kept at 20°C. and four at 30°C. To each jar was added 100 ml. of fresh, uninoculated, raw cream. Three jars in each temperature lot were inoculated with variable amounts of an *O. lactis* culture in sterile milk. One jar in each temperature lot was left without inoculation. Plate tests were at once made to determine the relative amounts of inoculum added in terms of live molds per ml. The plate count thus obtained is given in the "0 age" column in Table 4 for each of the three series. The fractional counts noted are averages of several plates. Series 2 and 3 were run in the same manner except that 10 jars were used for each series, one of which at each temperature (20° and 30°C.) was left uninoculated while the others were inoculated with variable numbers of *O. lactis* and these distributed equally to the two temperature conditions. Actually the amounts of inoculum added varied from 1 *O. lactis* per 10 ml. (.1 *O. lactis* per 1 ml.) to 16,000 per ml.

Cream was then added twice each day at 12 hour intervals and stirred in. Mold tests were made each day on the cream both by the MBB and the plate methods. At the end of 5 days the lots were churned by hand and mold mycelia counts were made on the butter.

The results of the plate count for each day on each lot are given in Table 4, while in Table 5 are given for comparison the results obtained on the fifth day by the MBB and plate count and by the mold mycelia method.

An examination of Tables 4 and 5 shows that while there was an increase in the number of molds in the 30° samples as the initial inoculum was increased, the increases in the 20° samples were negligible in all cases where the original count was less than 6 *O. lactis* per ml. On the basis of these experiments it would appear that for cream accumulated at 20°

TABLE 4.—*Effect of variable amounts of inoculation and temperature on mold development*  
(Numbers of *O. lactis* per ml. of cream)  
Cream stored at 20°C.

Age of cream (days)	SERIES 1						SERIES 2						SERIES 3					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
Original cream uninoculated	0	0	0	0	0	0	.2	0	0	0	0	0	0	0	0	.5	1	5
" " inoculated	.4	0	.5	0	0	0	.5	0	0	0	0	0	1	.5	0	.5	2	2
" " "	2	.3	.5	0	0	1	2	.5	.5	0	.5	1	12	4	5	19	70	870
" " "	6	4.0	5	34	230	2,500	13	3	5	2	5	48	930	195	260	1,140	6,450	16,000
							1,032	440	195	225	670	2,130	16,000	3,500	2,500	15,050	17,500	20,000

Cream stored at 30°C.*																		
Original cream uninoculated	0	0	0	0	0	0	.2	0	0	0	1	13	175	0	0	0	0	0
" " inoculated	.4	.7	16	36	87	190	.1	0	0	5	540	2,800	1	1	29	5,050	14,000	20,000
" " "	2	.3	30	22	17	300	2	2	3	1,120	19,000	31,500	12	11	2,225	27,500	50,000	74,500
" " "	6	15	8,050	19,000	17,500	21,500	13	9	435	14,050	39,500	49,500	930	365	53,000	185,000	225,000	285,000
							1,032	490	10,150	48,000	115,000	106,500	16,000	8,500	77,000	120,000	88,000	195,000

\* Yeasts occurred in numbers in Series 1 and 2 beginning with the second and third days, respectively, with the 30° temperature.

TABLE 5.—Comparison of plate count, MBB, and mold mycelia counts

PLATE COUNT (NUMBERS OF <i>O. LACTIS</i> PER ML.)			MBB AT END OF 5 DAYS (MM.)		MOLD MYCELIUM COUNT AT END OF 5 DAYS (%)	
INITIAL	END OF 5 DAYS		20°C.	30°C.	20°C.	30°C.
	20°C.	30°C.				
0	0	0	0	0	0	0
0	5	0	0	0	0	0
0.1	0	2,800	0	0	0	6
0.2	0	175	0	0	0	1
0.4	0	190	0	0	0	2
1.0	3	29,000	0	4.5	0	70
2.0	1	300	0	0	0	1
2.0	1	31,500	0	4.0	0	58
6.0	2,500	21,500	0	5.6	1	98
12.0	870	74,500	0	5.0	0	87
13.0	48	49,500	0	4.0	0	83
930.0	16,000	286,000	2.5	6.0	12	95
1,032.0	2,130	106,500	0	5.0	3	97
16,000.0	20,000	195,000	3.0	6.5	37	99

for periods of 5 days a mass inoculation is required before mold will develop appreciably and for cream held at 30°C. there must be in the neighborhood of one spore per ml. before growth is marked.

#### GENERAL DISCUSSION

A number of factors may influence the development of mold in cream. Attention has previously been called to the effect of fat test, size of shipment of cream, temperature, age of cream, use of clean utensils, and the effect of gravity separation of cream (2). All of these factors and possibly others are interrelated in their action in many instances. The data given in the present paper emphasize the importance of two basic factors that effect mold development; namely, the extent of the original inoculation and the temperature to which the cream is exposed. The presence or absence of large numbers of *O. lactis* spores in the first accumulations of cream appears to be a most important factor in determining whether mold will develop in gross amounts. For reasonable lengths of time and cooling even to 20°C. it is possible to keep cream free from excessive mold development provided large numbers of spores are not present and cream is added twice daily even when the additions are stirred in. Under the above conditions an occasional chance occurrence of *O. lactis* spores in cream does not appear to be of significance in initiating mold growth. On the other hand, failure to prevent the introduction of mold spores in large numbers at the beginning of the storage period cannot be overcome by cooling to 20°C. It is obvious that insanitary methods of production will tend to introduce an abundance of mold spores, thus contributing an initial high



inoculation in cream. Subsequent storage, even at comparatively low temperatures, will not prevent the development of mold in large quantities.

### SUMMARY

(1) In preliminary experiments the daily over-layering of heavy cream with fresh cream did not result in significant quantities of mold at temperatures in the neighborhood of 30°C., but when the daily additions of cream were stirred into the previous accumulation, there was a marked mold development at 30°C.

(2) Twice-a-day stirring of cream inoculated with mold did not of itself prevent mold development.

(3) In preliminary experiments cooling cream to 20°C. had a restraining action on mold development.

(4) The relative quantities of inoculum also had an influence on the extent of mold development.

(5) The combined effect of temperature and of relative quantities of inoculum was found to be important in evaluating mold results.

(6) Mold failed to develop in large amounts within 5 day periods when fresh cream was added twice a day and stirred in and a temperature of 20°C. maintained unless large quantities of *O. lactis* were present.

(7) In most instances cream treated similarly but held at 30°C. developed large amounts of mold when an initial contamination of 1 *O. lactis* or more per ml. was present.

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## OBSERVATIONS ON THE CHICK METHOD FOR THE ASSAY OF VITAMIN D

### I. RELATIVE ACCURACY OF GROUP AND INDIVIDUAL ASHING PROCEDURES AND RELATION OF CHICK WEIGHT TO PER CENT BONE ASH\*

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Several problems have arisen in connection with the present A.O.A.C. chick method for the assay of vitamin D.<sup>1</sup> The method provides that ash

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<sup>1</sup> *This Journal*, 22, 81 (1939); *Methods of Analysis*, A.O.A.C., 1940, 371.

determinations shall be made on the left tibiae by either group (composite) or individual ashing procedures. From this provision, it might be assumed that the results of the two ashing procedures would be equally accurate, and if so, the less laborious group ashing technic would be preferred. However, this assumption that group ashing is as accurate a procedure as individual ashing has not been examined in detail.

A second provision of the method is that all chicks weighing 100 grams or less at the end of the 3-week assay period shall be discarded. As this provision is based upon the fact that most of the chicks that show abnormality or disease, not related to vitamin D deficiency, will weigh 100 grams or less, it was assumed that the inclusion of any chicks within this weight range might lead to erroneous results. However, this assumption has not been investigated thoroughly.

### EXPERIMENTAL

A total of 12 separate experiments was conducted for the purpose of studying the two problems under discussion. In order to compare the results of the two ashing procedures, data were used from 9 experiments, each involving 4 groups. The groups consisted of approximately 23 chicks each, and they were fed respective levels of 0, 5, 10, and 15 units of vitamin D per 100 grams of feed. Individual ash determinations were made on the right tibiae of all chicks<sup>2</sup> regardless of weight. The composite ash determination for each group was calculated from the results obtained from the individual ash determinations by totaling the individual bone weights and totaling the individual ash weights.

Data from these same 9 experiments were also used in a comparison of the results of both ashing procedures for chicks weighing more than 100 grams. The differences in the results obtained for all chicks regardless of weight and for chicks weighing more than 100 grams were also compared.

Additional data to show the significance, if any, of this difference between the results for all chicks and for chicks weighing more than 100 grams were obtained from all 12 experiments involving a total of 50 groups of approximately 23 chicks each. These groups were fed levels of vitamin D varying from 0 to 30 units per 100 grams of feed. Individual ash percentages of the right tibiae were determined.

In order to study the exact relationship between chick weight and per cent bone ash, data were used from the 9 experiments previously mentioned. The per cent bone ash per chick weight was determined for each chick included in each of the 4 respective levels of vitamin D.

### DISCUSSION OF RESULTS

Comparisons of the results obtained from the individual and group (calculated) ashing procedures, involving all chicks regardless of weight

<sup>2</sup> In all experiments, chicks that showed abnormality or disease not related to vitamin D deficiency were discarded.

TABLE 1.—Comparisons of chick weight and per cent bone ash for all chicks regardless of weight and for chicks more than 100 grams

UNITS OF VIT. D PER 100 G. OF FEED	TOTAL NO. OF GROUPS	TOTAL NO. OF CHICKS		CHICK WEIGHT (g.)				PER CENT BONE ASH							
		ALL CHICKS REGARD- LESS OF WT.	CHICKS MORE THAN 100 g.	ALL CHICKS REGARD- LESS OF WT.		CHICKS MORE THAN 100 g.		ALL CHICKS REGARDLESS OF WT.				CHICKS MORE THAN 100 g.			
				AV.	AV. ST. DEV. <sup>2</sup>	AV.	AV. ST. DEV. <sup>2</sup>	AV. GROUP ASH (CALC.)	AV. INDIV. ASH	AV. <sup>2</sup> ST. DEV. (INDIV.)	AV. GROUP ASH (CALC.)	AV. INDIV. ASH	AV. <sup>2</sup> ST. DEV. (INDIV.)		
0	9	209	142	108	18	117	15	30.62	30.97	2.87	30.21	30.35	2.27		
5	9	218	177	123	26	130	20	34.14	34.48	3.66	33.73	33.83	3.24		
10	9	210	176	140	35	150	28	39.28	39.57	3.90	39.05	39.13	3.70		
15	9	201	157	142	41	157	32	42.96	43.25	2.90	42.76	42.86	2.80		

<sup>2</sup> The average standard deviation =  $\sqrt{\frac{\sigma^2 + \sigma^2 + \dots + \sigma^2}{N}}$  where "N" variances are averaged without weighting by the number of chicks in each group, and  $\sigma^2 = \frac{NSX^2 - (SX)^2}{N(N-1)}$  for "N" chicks. (The variance is the square of the standard deviation.)

and involving chicks weighing more than 100 grams are shown in Table 1 and Figure 1. Since all 4 lines in Figure 1 are essentially parallel throughout, it would appear that the group ashing procedure involving all chicks regardless of weight is as reliable as any of the procedures, and is to be preferred because of its greater simplicity. The 2 lines for group ash (calculated) do not coincide with the 2 respective lines for individual ash, but this would be expected since group ashing may be considered to be a form of weighting each individual per cent ash to obtain an average weighted per cent ash. That is, group ashing weights the per cent ash according to

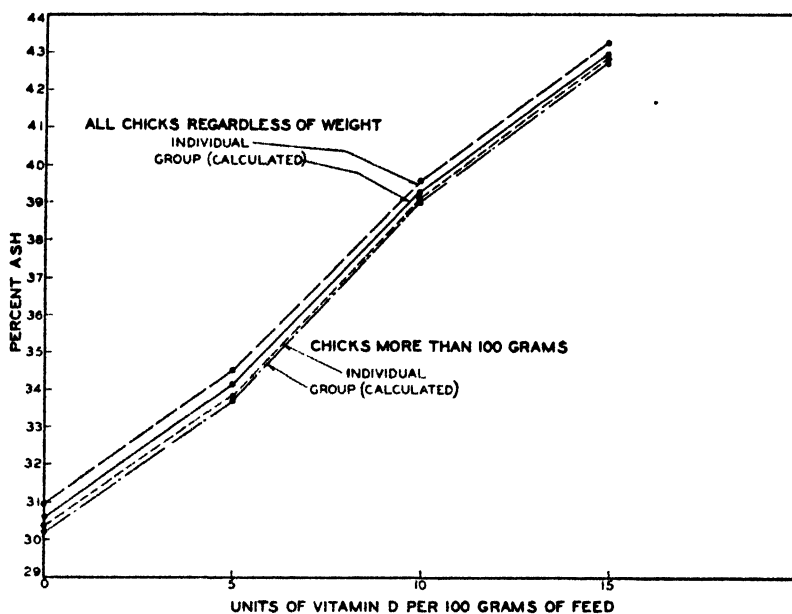


FIG. 1.—COMPARISONS OF INDIVIDUAL AND GROUP ASHING PROCEDURES INVOLVING ALL CHICKS REGARDLESS OF WEIGHT AND INVOLVING CHICKS WEIGHING MORE THAN 100 GRAMS. (Lines represent results from 9 separate experiments, each involving 4 respective levels of vitamin D.)

the weight of the bone, while an average of individual ash percentages gives the same significance to all bones.<sup>4</sup>

<sup>4</sup> This fact may be illustrated by a hypothetical example. Consider that a group of 20 chicks includes 15 that are normal except for vitamin D deficiency and 5 that are culls. The results of the 2 ashing procedures are given as follows:

NO. OF CHICKS	AV. BODY WT.	WT. OF MOISTURE-, FAT-FREE TIBIAE		WT. OF ASH		PER CENT ASH	
		AV.	TOTAL	AV.	TOTAL	GROUP	AV. INDIV.
15	grams 150	grams 0.50	grams 7.50	grams 0.17	grams 2.55	34	34
5	grams 60	grams 0.12	grams 0.60	grams 0.054	grams 0.27	45	45
Total 20			8.10		2.82	34.81	36.75

The results given in Table 2 show that the discarding of chicks that weigh 100 grams or less does make a significant difference in the per cent bone ash. This follows naturally from the fact that, at lower levels of vitamin D intake, the per cent bone ash is to some extent inversely proportional to the body weight of the chick, as will be shown below.

TABLE 2.—*Significance of difference between per cent bone ash for chicks weighing more than 100 grams, and for all chicks regardless of weight*

	NO. OF GROUPS	NO. OF CHICKS USED	AV. NO. CHICKS PER GROUP	MEAN DIFF. % ASH	t VALUE <sup>a</sup>	P <sup>b</sup>
Chicks more than 100 grams	50	885	18	0.56	7.76	<0.001
All chicks regardless of weight	50	1172	23			

<sup>a</sup> A test of significance of the difference between the two means:

$$t = \frac{M_1 - M_2}{\sqrt{\sigma_{M_1}^2 + \sigma_{M_2}^2}}, \text{ where } \sigma_{M_1}^2 = \frac{\sigma^2}{N}$$

The standard deviation,  $\sigma$ , for "N" chicks is calculated by:

$$\sigma = \sqrt{\frac{NSX^2 - (SX)^2}{N(N-1)}}$$

Where  $SX$  is the sum of the observations on "N" chicks and  $SX^2$  is the sum of the squares of observations on "N" chicks.

A complete explanation of these statistical procedures will be found in any textbook on statistics such as "Statistical Methods Applied to Experiments in Agriculture and Biology" by George W. Snedecor or "The Methods of Statistics" by L. H. C. Tippett.

<sup>b</sup> Probability showing significance of difference.  $P < 0.01$  indicates that a significant difference exists.

Individual per cent bone ash was plotted against chick weight for each level of vitamin D for each of the 9 experiments discussed under Table 1. It was evident that the relationship was linear and had no definite break or curvature upward at the 100 gram point, or any other point on the weight scale. Since all experiments yielded similar results, they were combined for each of the 4 respective levels of vitamin D fed. Straight lines were fitted by the method of least squares<sup>7</sup> to the scatter diagrams for each of the levels, as shown in Table 3 and Figure 2.

Since there is this linear relationship between per cent bone ash and chick weight, division of the chicks into 2 groups according to weight will produce a difference between the bone ash percentages of those 2 groups. As shown in Figure 2, such a difference will result in all cases, regardless of the chick weight taken as the dividing point. The lines in Figure 2 ex-

<sup>7</sup> In brief, for the equations given for the lines of Figure 2,  $X$  is chick weight and  $Y$  is per cent ash. The equation of a line is  $Y = a + bX$ , where "b," the slope, is calculated by means of the method of least squares,

$$b = \frac{NSXY - SXSY}{NSX^2 - (SX)^2}$$

and "a" is the "Y" intercept,

$$a = \bar{Y} - b\bar{X}, \text{ where } \bar{Y} = \text{mean of } Y, \text{ and } \bar{X} = \text{mean of } X.$$

The standard error of estimate showing the scatter about the line is calculated from the formula:

$$\sigma_{\text{est. } Y} = \sqrt{\sigma_y^2 - b^2\sigma_x^2},$$

where  $\sigma_y^2$  is variance of  $Y$  and  $\sigma_x^2$  is variance of  $X$ .

TABLE 3.—*Relationship of chick weight to per cent bone ash for 4 respective levels of vitamin D*

Units of vit. D/100 g. of feed	0	5	10	15
No. of chicks	208	217	210	201
Av. chick wt. (g.)	108	122	139	142
Range in chick wt. (g.): max.	166	208	237	239
min.	62	54	60	57
Av. per cent bone ash	30.99	34.72	39.92	43.35
St. dev. of per cent bone ash	2.94	4.07	4.36	3.02
Regression coefficient	-0.0364	-0.0272	-0.0166	-0.0141
Std. error of reg. coeff.	±0.0098	±0.0099	±0.0083	±0.0051
Standard error of estimate	2.85	4.00	4.32	2.97

tend over the entire range involved for each of the levels of vitamin D, as shown in Table 3. The 100-gram point, if extended upward, would cut off differing proportions of the ranges for each of the levels, excluding a greater proportion of the 0 unit level chicks than of the higher unit levels.

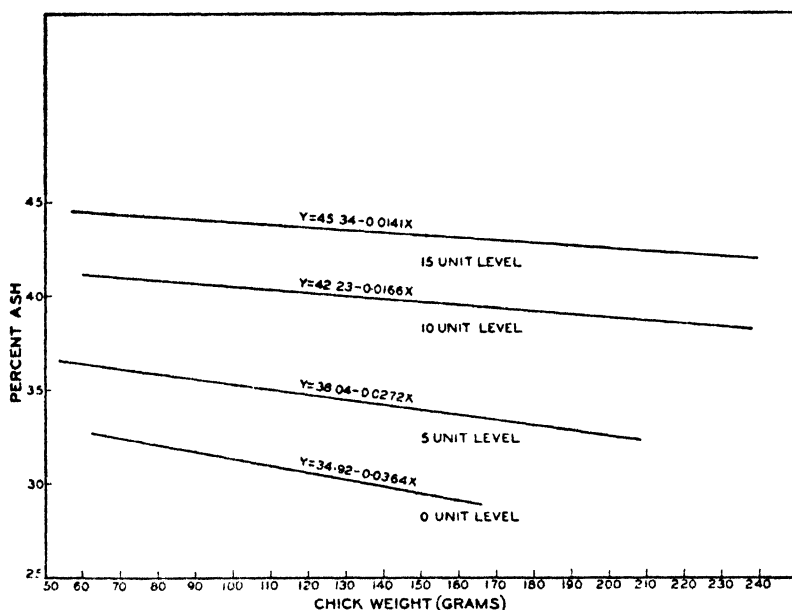


FIG. 2.—RELATIONSHIP OF CHICK WEIGHT TO PER CENT BONE ASH. (Lines represent results from 9 separate experiments, each involving 4 respective levels of vitamin D.)

Thus, the various levels would not be divided in the same proportion if one particular weight of chick was taken as the dividing line. Therefore, it would appear from the particular information given in Figure 2 that chicks should not be discarded on the basis of differences in body weight.

## SUMMARY

The chick method for the assay of vitamin D has been subjected to a critical study. The results obtained indicate that the group ashing procedure is as reliable as the more laborious and involved individual ashing procedure.

Statistical study of the data reveals a linear relationship between body weight and per cent bone ash of chicks for all levels of vitamin D studied. The data obtained indicate that body weight should not be made a basis for discarding chicks in the vitamin D assay procedure.

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EXTENSION OF THE RAPID VOLUMETRIC MICRO  
METHOD FOR DETERMINING ARSENIC

By C. C. CASSIL (Bureau of Entomology and Plant Quarantine,  
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Cassil and Wichmann (1) developed a volumetric micro method for determining arsenic in the range of 5-500 micrograms of arsenious oxide that involves evolution of arsine, absorption of the arsine in mercuric chloride, and the stoichiometric oxidation of the arsine to arsenic oxide. This method requires only 8-10 minutes for a single determination or 5 minutes when it is used in a routine manner on many samples. Details improving the method and collaborative results are given in a report by Cassil (2). Extensive routine analyses have shown that the method is entirely satisfactory with regard to speed, accuracy, and precision, but it is apparent that it would have wider application if the range were extended to 10 mg. of arsenious oxide. Other semimicro arsenic methods, such as the trichloride distillation followed by a bromate titration (4), specify that the sample be treated by a complete wet acid digestion, which procedure requires considerable time. A further study of the micro method described by Cassil and Wichmann has now shown that arsenic equivalent to 10 mg. of arsenious oxide can be determined by utilizing certain modifications without sacrificing speed, accuracy, or precision.

Smith (3) showed that quantities of arsenic equivalent to 10 mg. of arsenious oxide could be evolved as arsine, absorbed in mercuric chloride solution, and determined either by weighing the calomel formed in the reaction or by titrating the absorbed arsenious oxide after removing the calomel by filtration. The procedure requires at least one hour for the arsine evolution, 30 minutes' boiling to decompose the mercury arsenides into calomel and arsenious acid, and the additional time required for filtration, drying, and weighing the calomel, or titrating the arsenious acid.

The method presented in detail in this paper can be used for apple strip solutions (5) and for material that has been submitted to an acid diges-

tion. It may also be applicable to samples prepared in other ways. Preliminary work indicates that it is satisfactory for the analysis of insecticide arsenicals.

## ANALYTICAL PROCEDURE

### ISOLATION REAGENTS

- (a) *Hydrochloric acid*.—Use a C. P. concentrated acid that is As free.
- (b) *Zinc*.—Use a good grade of 20-mesh granulated Zn.
- (c) *Potassium iodide solution*.—Dissolve 15 grams of KI in water and dilute to 100 ml.
- (d) *Stannous chloride solution*.—Dissolve 40 grams of As-free  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  in 100 ml. of concentrated HCl.
- (e) *Absorbing solution*.—Dissolve 1.6 grams of  $\text{HgCl}_2$  (reagent grade) in about 60 ml. of water, heat if necessary to complete solution, cool, add 20 ml. of 2% aqueous U. S. P. gum arabic solution, and dilute to 200 ml.
- (f) *Lead acetate*.—Dissolve 10 grams of  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$  in 80 ml. of water, add sufficient acetic acid to have the solution just acid to litmus paper, and make to 100 ml. with water.

### TITRATION REAGENTS

- (g) *Buffer solution*.—Dissolve 10 grams of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  in water and dilute to 100 ml.
- (h) *Standard iodine solution*.—Prepare an approximately 0.05 *N* solution of  $\text{I}_2$  by dissolving 6.35 grams of pure  $\text{I}_2$  and 60 grams of KI in a small quantity of water, filtering, and diluting the filtrate to 1 liter. The excess KI is used to form the complex  $\text{K}_2\text{HgI}_4$  when added to the absorbing solution (e).
- (i) *Standard arsenic solution*.—Dissolve exactly 2.50 grams of standard  $\text{As}_2\text{O}_3$  in 25 ml. of a 20% NaOH solution. Saturate this solution with  $\text{CO}_2$  or neutralize with dilute  $\text{H}_2\text{SO}_4$ , and dilute to 1 liter with distilled water. 1 ml. of this solution contains 2.50 mg. of  $\text{As}_2\text{O}_3$ .
- (j) *Starch indicator*.—Mix about 1 gram of finely powdered soluble starch with cold water to a thin paste, add to about 200 ml. of boiling water, stirring constantly, and immediately discontinue heating. This solution can be preserved indefinitely by the addition to the reagent bottle of approximately 1 ml. of metallic Hg.

### APPARATUS

The apparatus is illustrated in Figure 1. Use a 125 ml. Erlenmeyer flask fitted with a 24/40 standard taper ground-glass joint for the generator, and attach this to an 18 cm. water-cooled condenser that has an 18/38 ground-glass joint on the upper end. Fit an adapter to the upper end of the condenser and connect the other end of the adapter to the delivery tube by means of a 10/30 ground-glass joint. (The end of the delivery tube is made of methyl methacrylate resin to prevent the sticking of the mercury arsenide on the inside. The baffle on the resin tube is an aid in stirring the solution.) Fill the adapter with a loosely packed wad of dry glass wool that has been previously saturated with reagent (f). (The glass wool, which acts as a scrubber to remove any  $\text{H}_2\text{S}$  that may be generated during the evolution, is replaced from time to time as it becomes black by the formation of  $\text{PbS}$ . It should not be used long enough to permit the black coloration to form beyond the center of the wad.) Construct the receiver so that the constricted end will be approximately 24 mm. in diameter and 90 mm. long, and allow the delivery tube to extend to the bottom. The upper end of the receiving tube is approximately 50 mm. in outside diameter and about 75 mm. long. The opening in the lower end of the delivery tube should not be over 2 mm. in diameter.





## ARSENIC ISOLATION PROCEDURE

Place in the generator flask a suitable aliquot from the solution to be analyzed (not to exceed 50 ml.) containing the equivalent of 0.50–10.0 mg. of  $\text{As}_2\text{O}_3$ , add sufficient concentrated  $\text{HCl}$  to make the total quantity of acid approximately 10 ml. and 5 ml. of the  $\text{KI}$ , dilute to 80–90 ml., and add about 1 ml. of the  $\text{SnCl}_2$  (after the dilution to prevent the reduction of  $\text{As}_2\text{O}_3$  to metallic arsenic). Place 20 ml. of the absorbing reagent in the receiver and connect it to the apparatus. Add 4–5 grams of the  $\text{Zn}$  to the generator flask and immediately connect the flask to the apparatus. Heat the solution in the generator flask nearly to boiling as rapidly as possible (1½–2 minutes) on a Bunsen burner or an electric hot plate and then discontinue heating for the remainder of the 5-minute evolution period.

NOTE: If  $\text{H}_2\text{SO}_4$  is present in the generator, the heating must be done more cautiously since frothing is experienced under these conditions.

## DETERMINATION

After the 5-minute evolution period, disconnect the receiver and the delivery tube, add from the buret through the delivery tube at least 7 ml. of the standard  $\text{I}_2$  solution (sufficient to give an excess of  $\text{I}_2$ ), and stir until a clear solution is obtained; add 10 ml. of the buffer reagent and titrate the excess iodine to a colorless end point with the  $\text{As}_2\text{O}_3$  solution, using starch as the indicator. If the end point is over titrated, add more  $\text{I}_2$  solution and again back titrate with  $\text{As}_2\text{O}_3$  solution.

$$\text{mg. As}_2\text{O}_3 = [\text{ml. I}_2 - (\text{ml. As}_2\text{O}_3 \times \text{factor})] - \text{blank} \times \text{I}_2 \text{ titer.}$$

NOTE: Since the blank is caused only by the arsenic in the evolution reagents and generally amounts to 1–2 micrograms, depending on the grade of reagents used, it is negligible when working with 0.05 *N* iodine. Considerable time can be saved by measuring all reagents, except the iodine and arsenious oxide solutions, in dropping bottles, or by any other convenient and appropriate method. The zinc may be added by means of a glass cup that holds 4–5 grams.

## DISCUSSION

In the proposed method, it is not necessary to subject pentavalent arsenic to a preliminary reduction because the potassium iodide is added to an acid solution containing the arsenic and this is heated nearly to boiling as quickly as possible. The arsine formed in the generator flask is swept through the apparatus by the excess hydrogen and absorbed quantitatively by the mercuric chloride. The volume of absorbing solution and the amount of gum arabic are so adjusted as to maintain a colloidal suspension of the mercuric arsenides and to afford an almost instantaneous oxidation of the mercury and arsenic. Actually the mercury and arsenic are oxidized by the iodine, but in effect this is the same as titrating arsine to arsenic oxide (1 As is equivalent to 8 I). Equations illustrating the reactions in this method are:

- (1)  $\text{As}_2\text{O}_3 + 6\text{H}_2 \rightarrow 2\text{AsH}_3 + 3\text{H}_2\text{O}$ ;
- (2)  $2\text{AsH}_3 + 12\text{HgCl}_2 + 3\text{H}_2\text{O} \rightarrow \text{arsenides} \rightarrow 12\text{HgCl} + \text{As}_2\text{O}_3 + 12\text{HCl}$ ;
- (3)  $12\text{HgCl} + 24\text{KI} \rightarrow 6\text{Hg}^\circ + 6\text{K}_2\text{HgI}_4 + 12\text{KCl}$ ;
- (4)  $6\text{Hg}^\circ + \text{As}_2\text{O}_3 + 8\text{I}_2 + 12\text{KI} + 2\text{H}_2\text{O} \rightarrow 6\text{K}_3\text{HgI}_4 + \text{As}_2\text{O}_3 + 4\text{HI}$ .

There is no evidence to indicate that any arsine is liberated from the solution in the generator during the first 30 seconds after the zinc is add-

ed, thus there is ample time to connect the generator to the apparatus. The first visible absorption of arsine in the mercuric chloride occurs in about 45 seconds, and approximately 75 per cent of the arsenic is evolved and absorbed between the first and second minute of evolution. The speed with which the arsine is evolved is illustrated for 250 micrograms and 10 milligrams of arsenious oxide in Figure 2.

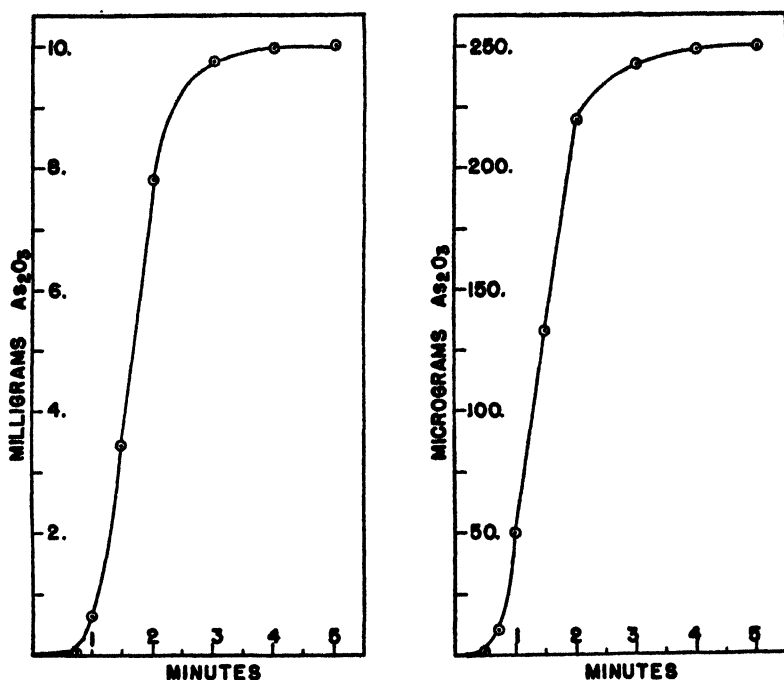


FIG. 2.—EVOLUTION CURVES SHOWING RATE AT WHICH THE ARSENIC IS EVOLVED FROM GENERATOR.

The optimum pH for this titration was determined for the micro procedure and found to be between 7 and 8.5. This pH also holds for the extended range but more buffer is used because the volume of solution in the titration vessel is approximately five times larger and a larger amount of hydrochloric acid is formed since the quantity of arsine absorbed is greater.

It was necessary to increase the concentration of gum arabic in the absorbing solution over that used in the micro method in order to prevent the flocculation and agglomeration of the larger quantities of mercury arsenides, and in this way, upon the addition of an excess of the iodine solution, an instantaneous oxidation is effected.

Interferences were thoroughly discussed in the paper describing the micro procedure (1). Antimony behaves in a manner similar to that of

arsenic and therefore must be separated before the arsenic is determined. Hydrogen sulfide would be a serious source of error but it is scrubbed out by the glass wool impregnated with lead acetate. The use in the generator of organic materials that might produce volatile reducing constituents not condensable or removable under the conditions of the

TABLE 1.—*Representative recoveries from routine analyses*

As <sub>2</sub> O <sub>3</sub> USED	As <sub>2</sub> O <sub>3</sub> FOUND	RECOVERY
<i>mg.</i>	<i>mg.</i>	<i>per cent</i>
0.50	0.509	101.8
0.50	0.503	100.6
1.00	1.01	101.0
1.00	0.99	99.0
2.00	1.98	99.0
2.00	1.97	98.5
2.50	2.45	98.0
2.50	2.47	98.8
5.00	4.91	98.2
5.00	4.98	99.6
7.50	7.35	98.0
7.50	7.38	98.4
10.00	9.91	99.1
10.00	9.97	99.7

method should be checked before results obtained on new products are accepted.

The buffer reagent must be added in the order given in the method. If it is added before the mercuric iodide complex is formed, even in the standardization of the iodine solution, erratic results may be obtained. An excess of the standard iodine solution (2-3 ml.) over that quantity required to precipitate and redissolve the mercuric iodide must be present in all titrations so as to have a sufficient quantity of free potassium iodide to form a suitable polyiodide solution, which is necessary for the stoichiometric oxidation.

Representative results of 14 recovery experiments are given in the table. The accuracy of this method as calculated from the results in the table is 99.26 per cent, and the precision is shown by a standard deviation of 1.14 per cent.

#### SUMMARY

The range of the rapid volumetric method for determining arsenic, previously considered as 5-500 micrograms, has been extended to 10 milligrams of As<sub>2</sub>O<sub>3</sub>. It is possible to complete a determination in less than 10 minutes after the necessary sample preparation. This method involves an arsine evolution, absorption in mercuric chloride-gum arabic

solution, and an iodine titration. Results presented show that the accuracy of the method is 99.26 per cent, with a standard deviation of 1.14 per cent.

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## BOOK REVIEW

**The Chemical Composition of Foods**, By R. A. McCANCE and E. N. WIDDOWSON. Chemical Publishing Company, Inc., New York. 1940. 150 pp. Price \$2.50.

This volume is the product of 14 years' intensive investigation in food chemistry. It presents a comprehensive treatment of the chemical composition, excluding the vitamins, of 541 food items commonly used in Great Britain. The analytical data reported here include values from previous publications which have been checked and corrected. Further, the results have been compared with works of other investigators, and any widely divergent values have been reexamined and discrepancies have been explained. One of the important features of this work is the system of analysis that the authors have developed by which they have determined quantitatively all of the important proximate and mineral constituents of the same foods. With the exception of calculated values for the composition of most of the cooked dishes and the sulfur of the cooked meats and fish, the data represent actual analyses performed in the authors' laboratory.

In their approach to the subject the authors have been guided by practical considerations. The foods have been analyzed not only in the raw state, but also as prepared for the table. In addition, the values on the "availability" of the P and Fe in some of the foods have been included. The authors recognize that the "available" phosphorus, that is the phosphorus not in the form of phytic acid and the "available" iron, the iron which reacts with  $\alpha\alpha'$ -dipyridyl may not be a true measure of their biological availability. However, in light of our present limited knowledge on the availability of the various nutritional constituents, these values may be of practical significance.

The foods have been classified into the following groups: dairy products, meat, poultry and game, fish, fruits, nuts, vegetables, etc. A complete index facilitates reference to individual foods.

This work is a valuable contribution to the information on the general composition of foods, offering indispensable quantitative data required for work involving detailed knowledge of the chemical composition of British foods in both the raw and cooked state. Since the foods and methods of preparation are, in general, comparable to those used in this country, this book should be of value as a highly practical handbook to physicians, nutritionists, dietitians, and nurses as well as research investigators in problems of human nutrition in the United States.—ELSA ORENT KEILES.



## MONDAY—MORNING SESSION

### REPORT ON ALCOHOLIC BEVERAGES

By J. W. SALE (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

This year the Referee received reports on proteolytic activity of malt, beer, heavy metals in beer, sulfur dioxide in wines, tannins in potable spirits, methanol in distilled spirits, and cordials and liqueurs. In addition to these reports, a paper on the photocolorimetric determination of tannins was submitted. Many of the reports, including the first five mentioned, contain extensive analytical data, mostly the results of collaborative work serving to support the recommendations made for future work.

Sufficient progress was made on a method for the determination of tannins in potable spirits and on four methods of analysis of cordials and liqueurs to warrant the adoption of these methods as tentative or official. This represents real progress. Recommendations for continuance of the investigations on the other subjects mentioned above and on those not reported on this year but recommended for further study last year were made. They will be found in the report of Subcommittee D.

No report on diastatic activity of malt was given by the associate referee.

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### REPORT ON PROTEOLYTIC ACTIVITY OF MALT

By STEPHEN LAUFER (Schwarz Laboratories, Inc., New York, N. Y.),  
*Associate Referee*

Since the presentation of the last report (1) by the Associate Referee, several papers have been published dealing with the proteolytic activity of malted grain.

Ayre and Anderson (2), correlating proteolytic activity with wort nitrogen and barley nitrogen fractions, measured the autolytic proteolytic activity by a modification of a method suggested by Northrup (3). In this procedure the material tested is subjected to autolytic digestion at 45° C. for 1 and 3 hours at pH 4.6. The difference between the amounts of non-protein nitrogen in the 1- and 3- hour digestions after precipitation with trichloroacetic acid was used to calculate the proteolytic activity.

Hildebrand (4) tested the proteolytic activity of a wide range of cereal products by the following methods:

- (1) The copper-precipitation method of Ritthausen (5) as modified by Blish (6), and Olsen and Bailey (7).
- (2) The Sörensen formol titration as modified by Samuel (8).
- (3) The gelation-rate procedure of Landis and Frey (9).
- (4) The viscometric method of Koch, Nelson, and Ehrnst (10).



Good agreement was obtained with Methods 1 and 2, and with Methods 3 and 4, although the results with both groups differed considerably. Hildebrand suggests that the first two methods measure dipeptidase and/or polypeptidase activity, whereas the physical methods measure essentially proteinase activity. Better differentiation between samples was obtained with Method 3 than with 4 and with Method 2 than with 1.

In a subsequent paper (11) Hildebrand compared the Landis and Frey rate-of-gelation, and the Ayre and Anderson autolytic-precipitation methods, using 12 samples of malted wheat. From the high degree of correlation between results as obtained by both methods, Hildebrand infers that the same type of activity is measured. He prefers the Ayre and Anderson procedure because of its comparative simplicity and convenience.

Attacking the problem of activity from an optical angle Landis (12) related the reduction in specific rotation of gelatin to the enzyme concentration of various materials acting on this substrate. While malt shows measurable activity, normal flours are too low in activity to be tested by this method.

During the past year the Associate Referee has continued the study of proteolytic activity along the lines recommended in his last report. It is believed that a procedure based on mash proteolysis has possibilities for estimating to some extent the proteolytic activity of malt. In order to elucidate the break-down of proteins resulting from mash proteolysis, a series of tests was conducted in which malt mashes were held at varying temperatures and pH conditions. Windisch, Kolbach, and co-workers (13) established these conditions of protein degradation for European malts, and Kelly (14) for a California malt made in England, but it was thought advisable to carry out similar tests for an American malt.

It should be mentioned at this point that Becker, Swanson, and Kruzic (15) performed tests on heat inactivation of proteolytic and other malt enzymes during mashing, and that these tests throw some additional light on mash proteolysis.

#### METHODS AND RESULTS

Mashes were conducted at various constant temperatures and pH ranges for 1 hour, and at a normal pH of 5.5 for 2 hours' duration as follows:

160 grams of malt, finely ground in the standard laboratory mill, and 550 ml. of acidified or alkalinized water, each previously brought to the required temperature, were mashed in and held at this temperature with frequent stirring for the period specified. The mashes were immediately chilled in ice in order to stop further enzyme action, and the weight of the mash was made up with cold distilled water to a total of 760 grams. Filtration was conducted in the cold, and the worts were kept cold until ready for analysis. The hydrogen ion concentration was adjusted by means of dilute  $H_2SO_4$  and  $NaOH$ , and the pH was estimated colorimetrically and by means of the quinhydrone electrode on the filtered wort on completion of the mash. The

extract, soluble nitrogen, permanently soluble nitrogen, and formol nitrogen were determined by the methods indicated in the first report (16).

By soluble nitrogen is meant the nitrogen rendered soluble either during mashing or during extraction in the cold. For determination of permanently soluble nitrogen the worts were boiled down to half the volume and kept in ebullition for 2 hours, boiling water being added frequently to keep the volume constant; then they were brought to the original volume at 20° C. and filtered through folded filter paper. A nitrogen determination was made on the filtrate, which was also used for estimation of formol nitrogen by the method described in Pawlowski-Doemens (17).

The nitrogen figures were calculated to the basis of 100 grams of dry malt by multiplying the values found in the respective worts by  $Y/(E \times S)$ , in which  $S$  indicates the specific gravity,  $E$  the corresponding extract in per cent (Plato) of the wort examined, and  $Y$  the yield of extract, dry basis, as computed from the former values and the moisture content of the malt.

The following formula was used for calculation of the yield of extract, dry basis:

$$Y = \frac{E(375 + M) 100}{(100 - E) (100 - M)},$$

in which  $M$  indicates per cent moisture.

The results are presented in Tables 1-3 and Figures 1-6.

Some irregularities will be apparent in the results, owing to experimental errors inherent in the procedures used. This applies particularly to mashing, filtration, effect of various amounts of sodium and sulfate ions, and determination of permanently soluble and formol nitrogen. Similar difficulties were encountered by other workers in this field (Windisch and Kolbach, Kelly).

#### DISCUSSION

The values presented in Table 1 and in Figures 1-3 indicate proteolysis occurring in malt mash conducted for 1 hour at  $pH$  levels ranging from 4.5 to 6.6 and at temperatures ranging from 7° to 75° C., as measured by determinations of soluble, permanently soluble, and formol nitrogen. These tests were carried out for one sample of malt (No. 1). The results of mash proteolysis occurring in malt mash conducted for 2 hours at a normal  $pH$  of 5.5 and at various temperatures are shown for three samples of malt in Table 2 and in Figures 4-6. These results include the values for soluble, permanently soluble, and formol nitrogen pre-existing in the malt prior to mashing. If the figures obtained in the mash conducted at 7° C. are deducted from the results obtained at other temperatures, the balance represents proteolysis occurring during mashing only at various temperatures and  $pH$  levels. These values are presented for 1 hour mash in Table 3.

The results obtained in the present investigation differ somewhat from those obtained by other workers. Windisch, Kolbach, and co-workers (13) established the following optimal conditions for mash proteolysis on a European malt:

Temperature Optima

	Perm. Sol. N. °C.	Formol N °C.
1 hour mash	57-59	52-56
2 hour mash	56-58	48-52

pH Optima

Temperature °C.	Perm. Sol. N	Formol N
30	4.3-4.7	4.3-4.7
50	4.3-4.7	4.4-4.8
70	4.9-5.3	4.6-5.0

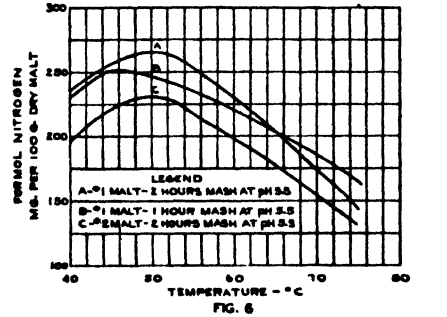
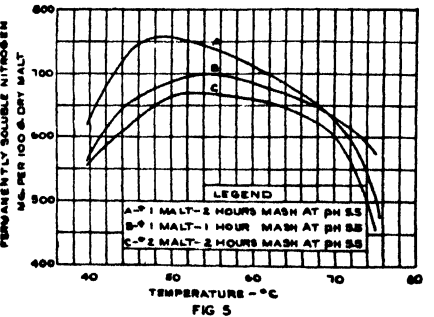
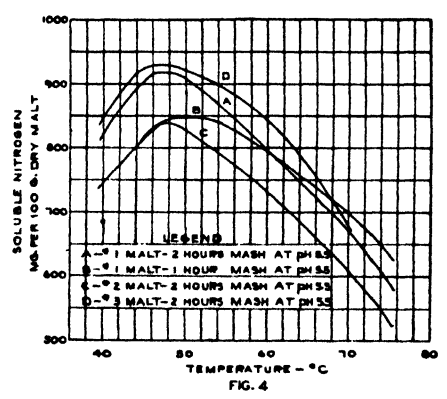
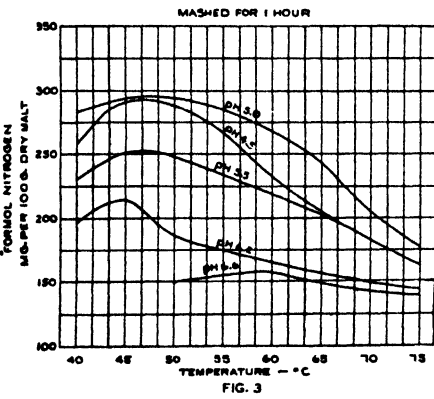
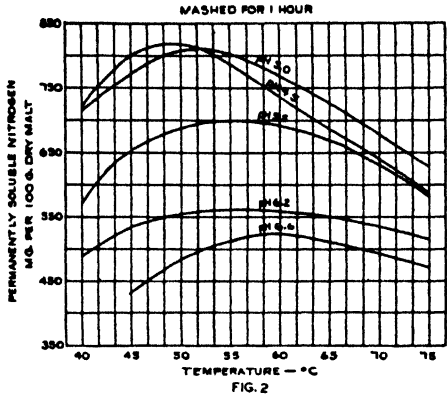
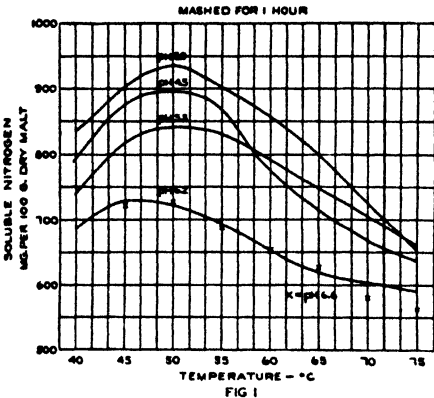


TABLE 1.—*Effect of temperature and pH on protein degradation of malt mash*  
(160 parts malt + 600 parts H<sub>2</sub>O)

Moisture (%) pH	Time of mash: 1 hour				Total N of dry malt: 2.203 %			
	7°C.				40°C.			
9.0 4.5	5.0	5.5	6.2	6.6	8.5 4.5	5.0	5.5	6.2
Sp. gr. at 17.5°C.								
1.01851	1.01904	1.01810	1.01807	—	1.02731	1.03002	1.02451	1.02144
Extract Plato								
4.96	4.84	4.61	4.60	—	6.88	7.56	6.19	5.43
Soluble N (mg./100 ml.)	125.0	130.0	141.0	—	181.0	190.0	170.0	158.0
Perm. Sol. N (mg./100 ml.)	98.0	93.0	91.0	—	165.0	166.0	130.0	112.0
Formol N (mg./100 ml.)	35.0	32.0	31.0	—	59.0	64.0	53.0	45.0
Dry Malt Basis (%)								
22.0	21.5	20.4	20.4	—	31.0	34.3	27.7	24.1
Soluble N	0.544	0.557	0.566	0.613	0.793	0.836	0.741	0.686
Perm. Sol. N	0.465	0.426	0.405	0.396	0.723	0.730	0.567	0.486
Formol N	0.152	0.152	0.139	0.135	0.258	0.282	0.231	0.195
50°C.								
7.4 4.5	5.0	5.5	6.2	6.6	6.8 4.5	5.0	5.5	6.2
Sp. gr. at 17.5°C.								
1.03138	1.03229	1.03012	1.02801	1.02248	1.03951	1.05045	1.04475	1.04681
Extract Plato								
7.89	9.57	7.58	7.06	5.69	9.86	12.47	11.12	11.61
Soluble N (mg./100 ml.)	205.0	213.0	184.0	170.0	199.0	—	190.0	157.0
Perm. Sol. N (mg./100 ml.)	188.0	184.0	157.0	113.0	186.0	—	158.0	—
Formol N (mg./100 ml.)	66.0	—	55.0	35.0	64.0	—	33.0	39.0
Dry Malt Basis (%)								
35.6	43.7	33.9	31.4	24.9	44.8	58.4	51.3	56.8
Soluble N	0.895	0.937	0.842	0.728	0.870	—	0.838	0.700
Perm. Sol. N	0.818	0.810	0.681	0.536	0.813	—	0.701	—
Formol N	0.287	—	0.239	0.186	0.280	—	0.234	0.173
65°C.								
7.0 4.5	5.0	5.5	6.2	6.6	7.0 4.5	5.0	5.5	6.2
Sp. gr. at 17.5°C.								
1.05549	0.05948	1.05940	1.05719	1.05586	1.05721	1.06053	1.06134	1.06028
Extract Plato								
13.65	14.59	14.56	14.06	13.74	14.06	14.83	15.03	14.77
Soluble N (mg./100 ml.)	159.0	179.0	170.0	139.0	148.0	155.0	158.0	133.0
Perm. Sol. N (mg./100 ml.)	152.0	—	149.0	118.0	142.0	147.0	—	121.0
Formol N (mg./10 ml.)	46.0	55.0	45.0	34.0	43.0	45.0	41.0	34.0
Dry Malt Basis (%)								
64.9	70.2	70.0	67.2	65.4	67.2	71.5	72.7	71.2
Soluble N	0.717	0.813	0.772	0.619	0.669	0.705	0.720	0.605
Perm. Sol. N	0.686	—	0.676	0.533	0.642	0.689	—	0.551
Formol N	0.207	0.250	0.204	0.154	0.104	0.205	0.187	0.155
70°C.								
7.2 4.5	5.0	5.5	6.2	6.6	7.2 4.5	5.0	5.5	6.2
Sp. gr. at 17.5°C.								
1.05561	1.06027	1.06037	1.05987	1.05916	1.05561	1.06027	1.06037	1.05987
Extract Plato								
13.65	14.59	14.56	14.06	13.74	14.06	14.83	15.03	14.77
Soluble N (mg./100 ml.)	159.0	179.0	170.0	139.0	148.0	155.0	158.0	133.0
Perm. Sol. N (mg./100 ml.)	152.0	—	149.0	118.0	142.0	147.0	—	121.0
Formol N (mg./10 ml.)	46.0	55.0	45.0	34.0	43.0	45.0	41.0	34.0
Dry Malt Basis (%)								
65.3	71.4	71.5	70.6	68.9	65.3	71.4	71.5	70.6
Soluble N	0.637	—	0.634	0.592	0.637	—	0.634	0.592
Perm. Sol. N	0.588	—	0.584	0.519	0.588	—	0.584	0.519
Formol N	0.163	0.178	0.164	0.146	0.163	0.178	0.164	0.146

TABLE 2.—*Effect of various temperatures at normal pH (5.5) on protein degradation of malt mashes*  
(160 parts malt + 600 parts H<sub>2</sub>O)

Total N of dry malt: No. 1 2.203% No. 2 2.085% No. 3 2.223%

	40°C.			50°C.			60°C.		
	1 HR. MASH	2 HR. MASH	MALT 3	1 HR. MASH	2 HR. MASH	MALT 3	1 HR. MASH	2 HR. MASH	MALT 3
Moisture (%)	8.5	8.8	4.4	7.44	8.8	4.3	6.9	8.5	4.3
Sp. gr. at 20°C.	1.02446	1.02788	1.02594	1.03010	1.03767	1.03871	1.05607	1.05830	1.06025
Extract Plato	6.19	7.04	7.42	7.58	9.43	9.68	13.82	14.34	14.80
Soluble N (mg./100 ml.)	170.0	186.0	203.0	194.0	203.0	218.0	171.0	172.0	183.0
Perm. Sol. N (mg./100 ml.)	130.0	142.0	130.0	157.0	169.0	—	152.0	152.0	159.0
Formol N (mg./100 ml.)	53.0	53.0	55.0	55.0	59.0	—	—	51.0	53.9
<i>Dry Malt Basis (%)</i>									
Extract	27.7	31.9	31.8	33.9	43.8	42.5	65.8	70.2	68.9
Soluble N	0.741	0.820	0.844	0.842	0.909	0.922	0.771	0.795	0.847
Soluble N (% of Malt N)	33.6	37.2	38.0	38.2	41.3	41.5	35.6	36.1	38.1
Perm. Sol. N	0.567	0.626	0.607	0.681	0.757	0.663	0.686	0.702	0.698
Perm. Sol. N (% of Malt N)	25.7	28.4	27.3	30.9	34.4	—	31.1	31.9	31.4
Formol N	0.231	0.234	0.229	0.239	0.264	—	—	0.236	0.233
Formol N (% of Malt N)	10.5	10.6	10.3	10.9	12.0	—	—	10.7	10.5
Formol N (% of Perm. Sol. N)	40.7	37.4	37.7	35.1	34.9	—	—	33.6	33.4
<i>Dry Malt Basis (%)</i>									
Extract	7.0	8.8	4.6	7.2	8.9	7.4	—	—	—
Moisture (%)	7.0	8.8	4.6	7.2	8.9	7.4	—	—	—
Sp. gr. at 20°C.	1.06125	1.05978	1.06009	1.06022	1.05873	1.05880	—	—	—
Extract Plato	15.03	14.69	15.39	14.79	14.44	14.46	—	—	—
Soluble N (mg./100 ml.)	158.0	147.0	154.0	147.0	123.0	115.0	—	—	—
Perm. Sol. N (mg./100 ml.)	—	138.0	148.0	138.0	104.0	101.0	—	—	—
Formol N (mg./100 ml.)	41.0	37.0	39.0	36.0	31.0	30.0	—	—	—
<i>Dry Malt Basis (%)</i>									
Extract	72.7	72.5	72.4	71.5	71.1	69.8	—	—	—
Soluble N	0.720	0.684	0.681	0.634	0.571	0.524	—	—	—
Soluble N (% of Malt N)	32.7	31.1	30.6	28.9	25.9	25.1	—	—	—
Perm. Sol. N	—	0.642	0.654	0.584	0.484	0.461	—	—	—
Perm. Sol. N (% of Malt N)	—	29.1	29.4	26.5	22.0	22.1	—	—	—
Formol N	0.187	0.172	0.172	0.164	0.144	0.137	—	—	—
Formol N (% of Malt N)	8.5	7.8	7.4	7.7	6.5	6.6	—	—	—
Formol N (% of Perm. Sol. N)	—	26.9	26.3	23.1	29.8	29.7	—	—	—

Kelly (14), on the other hand, found for a California malt produced in England temperature optima of 50°–55° C., which are somewhat lower than those obtained on the Continent:

<i>Temperature</i> °C.	<i>Sol. N</i>	<i>pH Optima</i> <i>Perm. Sol. N.</i>	<i>Formol N</i>
40	4.4–4.6	4.4–4.6	4.4–5.0
55	4.6–5.0	4.6–5.0	4.4–5.2
65	4.8–5.2	5.0–5.2	4.6–5.2

The pH optimum shows a tendency to shift towards the alkaline side with rise in temperature.

In the present tests the pH optima were found to be 4.5–5.0 at temperatures of 45°–50° C. for soluble nitrogen, 45°–55° C. for permanently soluble nitrogen, and 45°–50° C. for formol nitrogen. It is apparent that these results differ somewhat from those obtained by the other investigators, but these differences probably are due to the different malts used and also to difference in experimental procedure.

The values for proteolysis obtained for the three samples of malt (Table 2 and Figures 4–6) are of particular interest, as they demonstrate differences in extent of proteolysis from malt to malt. Thus malt No. 1 (curves A and B) definitely is more subject to proteolysis under the same conditions than is malt No. 2 (curve C).

TABLE 3 — *Effect of temperature and pH on protein degradation of malt mash*  
(Nitrogen Increase (mg./100 grams dry malt) for 1 hour mash after deduction of values for control mash (1 hour at 7° C.)

		<i>Soluble Nitrogen</i>							
		40°	45°	50°	55°	60°	65°	70°	75°
<i>pH</i>	4.5	249	333	352	326	232	173	125	93
	5.0	279	344	380	—	303	256	148	—
	5.5	175	254	276	272	205	206	154	68
	6.2	73	116	104	87	—	6	—8	—21
		<i>Permanent Soluble Nitrogen</i>							
		40°	45°	50°	55°	60°	65°	70°	75°
<i>pH</i>	4.5	258	337	353	348	271	221	177	123
	5.0	304	347	384	—	—	—	243	—
	5.5	162	257	276	296	281	271	—	179
	6.2	90	153	140	—	162	137	155	123
		<i>Formol Nitrogen</i>							
		40°	45°	50°	55°	60°	65°	70°	75°
<i>pH</i>	4.5	106	140	135	128	80	55	42	11
	5.0	130	141	—	—	117	98	53	26
	5.5	92	112	100	95	—	65	48	25
	6.2	60	80	51	38	—	19	20	11

Windisch, Kolbach, and co-workers (13) advise against using the soluble nitrogen figure for estimating the extent of proteolysis, owing to the fact that a considerable amount of nitrogen is coagulated by mashing at higher temperatures; therefore, these authors employ the value of permanently

soluble nitrogen for this purpose. However, if the proteolysis is carried at relatively low temperatures, such as 40°–50° C., for 1–2 hours, there should be little coagulation of nitrogen, and this procedure of soluble nitrogen could be used for estimating proteolysis. Furthermore, a pH level of 5.0 could be selected, which is close to the optimum pH for proteolysis and also not far from the pH levels prevailing in normal brewers' mashes (5.2–5.4).

For estimating the soluble nitrogen pre-existing in the malt, a cold mash, or a mash conducted under the same conditions as the main mash but only for half an hour, could be used. In addition, the suggested procedure offers possibilities for determining in one operation both the proteolysis and diastasis of malts.

It is recommended that further work be done on mash proteolysis of malt.

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No report on malt extract in malt was given by the associate referee.

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No report on malt adjuncts was given by the associate referee.

#### REPORT ON BEER

By HUGO W. ROHDE (Jos. Schlitz Brewing Co.,  
Milwaukee, Wis.), Associate Referee

Collaborative work was not done during 1940. In his 1939 report the Associate Referee on Beer made a number of suggestions and recommendations to the Referee on Alcoholic Beverages, and it is gratifying to note

that they were approved. The A.O.A.C. methods for the determination of the important ingredients of beer are almost universally accepted and followed at present, but minor modifications are sometimes introduced. The third revised edition of "Methods of Analysis," issued by the American Society of Brewing Chemists during the latter part of September, 1940, now includes methods for the analysis of beer comprising 14 separate determinations, excluding instructions for preparing the sample. Three of the determinations involve calculations from the analytical data obtained. The methods do not vary from those previously presented before this Association.

During the year, Philip P. Gray, Chairman of the Technical Committee on Beer Analysis, American Society of Brewing Chemists, sent two samples of beer wort to 15 collaborators, and after these samples had passed through the customary cellar operations the finished beers were submitted to the analysts. After certain determinations had been made, the wort was fermented with a standard commercial brand of yeast, and the following determinations were made on the fermented laboratory beer: specific gravity, alcohol, real extract, protein, and total acidity by alkali titration and by potentiometric titration. The original extract was calculated from the data obtained by analysis. This excellent collaborative study showed a remarkably close agreement in results.

In 1938, the writer was appointed Associate Referee on Beer, and, as has been reported previously, submitted samples of beer to 14 collaborators. There is no purpose in repeating this work, as experience has demonstrated clearly that very satisfactory comparative results are obtained by trained analysts.

The Alcoholic Beverages Subcommittee has suggested a collaborative study of the following determinations relating to beer: (a) Extract in Original Wort, (b) Real Degree of Fermentation, (c) Total Acidity, (d) Dextrins, (e) Direct Polarization, (f) Pasteurization, (g) Chlorides, and (h) H-ion Concentration.

With reference to these suggestions, the Associate Referee comments and recommends as follows:\*

(a) *Extract in Original Wort.* In addition to the formula—

$$O = \frac{(A \times 2.0665) + E}{100 + (A \times 1.0665)} \times 100,$$

contained in *Methods of Analysis, A.O.A.C.*, and accepted as the standard formula both here and abroad, a second formula, more simple and convenient, was suggested in 1939:

$$O = 2A + E \pm \text{correction, in which}$$

$O$  = extract of original wort;

$A$  = per cent of alcohol by weight (g. per 100 g. of beer); and

$E$  = real extract.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 63 (1941).



The "correction" is given in the publication by Pawlowski-Doemens, "Die Brautechnischen Untersuchungs—Methoden," Fifth Edition, 1938, and also in the "Tables Related to Determinations on Wort and Beer," published by the American Society of Brewing Chemists, 1940 Edition. It has been suggested that this table be included in the next edition of *Methods of Analysis*, A.O.A.C. The collaborative work referred to previously verified the fact that both formulas give identical results.

(b) *Real Degree of Fermentation*.—The real extract is obtained by analysis, and the extract of the original wort is obtained by calculation from the data on alcohol and real extract by the following formula:

$$\frac{\text{Extract in original wort—real extract}}{\text{Extract in original wort}} \times 100.$$

This value is of importance in so far as it gives information concerning abnormal brew house or cellar operations.

(c) *Total Acidity*.—The method recommended for the determination of total acidity was adopted as tentative, *This Journal*, 22, 68 (1940). This method gives very satisfactory results and has been included, also, in "Methods of Analysis," American Society of Brewing Chemists.

(d) *Dextrins*.—The Associate Referee recommends that the subject of dextrins be studied further.

(e) *Direct Polarization*.—The polariscope is not suitable for the examination of beer, owing to the fact that optical rotation is influenced in a varying degree by the different sugars, dextrins, and other organic substances. The results obtained by direct polarization are of no practical value.

(f) *Pasteurization*.—Proper pasteurization insures biological stability of beer. Standard procedures are followed in routine operations, insuring destruction of microorganisms or inhibition of further development. The only safe and reliable methods for verifying biological soundness of beer are incubation at temperatures favorable for microscopic growth, or plating in culture media, followed by the usual bacteriological examination.

(g) *Chlorides*.—Chlorides are normally present in beer in infinitesimal quantity; they have their origin in the brewing water and brewing materials. The addition of sodium chloride to the wort—a practice that was followed occasionally several generations ago—is now obsolete. Accidental leakage of brine from cooling systems may increase the salt content, but this would give rise to an unsalable product. In the opinion of the Associate Referee, studies will be made on chlorides if this work is desired by the committee.

(h) *H-ion Concentration*.—The electrometric method for pH determination has been found very satisfactory, giving results within experimental error. It has been accepted as tentative, and it is here recommended that it be made official.

The following subjects are suggested for future study: total acidity by potentiometric titration, determination of dextrans, sulfur dioxide, and metals such as iron, tin, and copper. The studies on metals were assigned to another committee.

## REPORT ON HEAVY METALS IN BEER

By WILLIAM H. HARRISON (Continental Can Company,  
Chicago, Ill.), *Associate Referee*

The work done this year on methods for the determination of heavy metals in beer was confined to methods for the determination of iron in beer.

Since the iron content of various beers ranges from 0.1 p.p.m. to slightly over 1.0 p.p.m., it is very important that any satisfactory method for this determination be very accurate for these small quantities. The literature contains numerous procedures for the determination of iron in foodstuffs, all of which have been considered by the Associate Referee in regard to their applicability to beer.

These methods vary in the manner of destruction of organic matter, and in the ultimate means of estimating the iron present. Ashing and digestion with certain oxidizing acids, such as mixtures of sulfuric and nitric or perchloric and nitric acids, are common methods for the destruction of organic matter. The iron is commonly determined by such reagents as potassium thiocyanate (1), potassium ferrocyanide (2), alpha alpha dipyridyl (3), orthophenanthroline (4) 8-hydroxy quinoline (5), and other organic reagents. Excellent results have been reported on methods specifying an amyl alcohol extraction of the iron thiocyanate from aqueous solution that tends to intensify and concentrate the color (6, 7). Methods wherein the color is developed in the beer itself by the use of alpha alpha dipyridyl or potassium thiocyanate are not believed to give the accuracy desired.

In choosing a method most suitable for the determination of iron in beer the Associate Referee kept in mind two main factors, simplicity and accuracy. The best method for the destruction of organic matter was found to be a wet ashing procedure specifying perchloric and nitric acids. The hazard connected with the use of perchloric acid is eliminated by the predigestion of the sample by the nitric acid in the mixture and by the small quantity of perchloric acid used. This method of ashing, which was found to be very rapid, seems to eliminate entirely the error caused by the formation of iron pyrophosphate during dry ashing procedures. It is very important, however, that the acids used in the digestion mixture be practically iron free. Redistilled nitric acid and double-distilled perchloric acid are recommended. Results that were obtained on dry ashed samples

were satisfactory but not so consistent as those obtained on similar samples wet-ashed.

The method selected for the determination of iron was that of Winsor (8), in which the iron thiocyanate color is developed in an acid medium of approximately 75 per cent methoxyethanol (methyl cellosolve). The color is quite stable in this medium and is approximately 85 per cent more intense than in a water medium.

#### METHOD FOR IRON IN BEER

##### REAGENTS

(a) *Nitric acid*.—Redistil through Pyrex and store in Pyrex bottles.

(b) *Perchloric acid*.—Double-vacuum distilled 72%  $\text{HClO}_4$ . (May be obtained from G. Frederick Smith Chemical Company, Columbus, Ohio.) Store in Pyrex bottles.

(c) *Methyl cellosolve*.—Dissolve 80 grams of  $\text{NH}_4\text{SCN}$  in methyl cellosolve and dilute to 2 liters with the same solvent. A faint pink color may be noticeable after the reagent has been prepared, but this will fade upon standing.

(d) *Standard iron solution*.—Contains the equivalent of 0.01 mg. of ferric iron per ml. Add small quantity of bromine water.

##### PREPARATION OF STANDARDS

Make up standards by using water, concentrated  $\text{HCl}$ , and the methyl cellosolve in the following proportions:

	ml.
Standard iron solution	X
Water	5-X
Concentrated $\text{HCl}$	1.25
Methyl cellosolve	18.75

##### DETERMINATION

Pipet 25 ml. of thoroughly degassed beer into a 100 ml. extraction flask (Pyrex No. 5160) and boil down to a thick sirup, slightly charred. (It is necessary to use a flask with a rather small base so that 2 ml. of  $\text{HClO}_4$ , which remains at the end of the digestion, will cover the entire bottom surface of the flask.) Add 20–25 ml. of the  $\text{HNO}_3$  and 2 ml. of the  $\text{HClO}_4$ . Cover the flask with a watch-glass and heat gently until the initial reaction begins, at which stage a fairly vigorous boiling with the evolution of brown oxides of nitrogen takes place. After the reaction has subsided again heat the contents of the flask to a slow boiling and continue the boiling until all the  $\text{HNO}_3$  is driven off. (With certain beers, or when too rapid boiling is used, this procedure may not complete the oxidation. In this case the solution rapidly turns brown, or even black, depending upon how much material remains unoxidized. A few additional ml. of  $\text{HNO}_3$  at this stage will produce a clear but not colorless solution (brown).) Continue the heating to expel all the  $\text{HNO}_3$ , as evidenced by the evolution of copious fumes of  $\text{HClO}_4$ . (On cooling, the residue should be colorless or at most a pale yellow. If this is not the case, further heating with small additions of  $\text{HNO}_3$  and  $\text{HClO}_4$  should be used.)

After slight cooling, add 5 ml. of distilled water and warm the mixture until the precipitated salts are in solution. Allow the solution to cool to room temperature and add 18.75 ml. of the ammonium thiocyanate-methyl cellosolve reagent. Immediately after the color is developed, place the flasks and standards in a pan of circu-

lating cold water, since heat causes a slow fading of the red color. Compare the color produced in the sample with that of a standard of almost similar color intensity by means of a colorimeter or photometer.

In testing the accuracy of the method, analyses were made of samples of beer to which known quantities of iron as ferric chloride had been added. Table 1 shows the results.

TABLE 1.—*Recoveries of added iron*  
(Weight of beer 25 grams.)

NO.	IRON ADDED	IRON FOUND	IRON RECOVERED	
	mg.	mg.	mg.	per cent
1	—	0.017	—	—
2	—	0.017	—	—
3	0.005	0.022	0.005	100
4	0.005	0.021	0.004	80
5	0.010	0.028	0.011	110
6	0.010	0.026	0.009	90
7	0.015	0.034	0.017	113
8	0.015	0.035	0.018	120
9	0.020	0.036	0.019	95
10	0.020	0.035	0.018	90
11	0.025	0.043	0.026	104
12	0.025	0.042	0.025	100
Average				100

A comparison was made between the wet-ashing procedure described previously and the same method including a dry-ashing procedure. With the wet-ashing procedure, 50 ml. of beer was evaporated to dryness in a 200 ml. casserole and ignited to a carbon-free ash in a muffle at 1000° F., oxygen being used as an accelerator. The ash was then dissolved in a mixture of approximately 2 ml. of concentrated hydrochloric acid and distilled water, warmed to facilitate solution, and filtered. The filtrate was evaporated to dryness, after which 1.25 ml. of concentrated hydrochloric acid was added and the color developed as described above. Results obtained by the two procedures on the same lot of beer are shown in Table 2.

In order to test the method further, two lots of beer that were known to differ widely in iron content were obtained. The individual bottles from each lot were opened, and the beer was poured into a large carboy, where it was mixed, rebottled (aluminum spot crowns used), and repasteurized. Samples from the two lots, together with sufficient quantities of double-distilled perchloric acid and methyl cellosolve, were sent to collaborators, who were requested to analyze the samples by the wet-ashing procedure outlined here and by any other methods they might desire, for the purpose of comparison. Those who collaborated in this program included Wahl-

Henius Institute, Chicago, Ill.; Schlitz Brewing Company, Milwaukee, Wis.; American Can Company, Maywood, Ill.; Pacific Chemical Laboratories, San Francisco, Calif.; Wallerstein Laboratories, New York City; E. A. Siebel Company, Chicago, Ill.; Anheuser-Busch Inc., St. Louis, Mo.; Siebel Institute of Technology, Chicago, Ill.; Barbey's Inc., Reading, Penn.; and Continental Can Company, Chicago, Ill. The results reported by these collaborators are shown in Table 3.

TABLE 2.—*Ashing procedure results*  
(p.p.m. Iron found.)

NO.	DRY-ASHING PROCEDURE	WET-ASHING PROCEDURE
1	0.70	0.68
2	0.67	0.66
3	0.65	0.64
4	0.62	0.66
5	0.62	0.68
6	0.59	0.64
Average	0.64	0.66

#### COMMENTS BY COLLABORATORS

*Collaborator No. 2.*—In Test A the color of one sample was fugitive, but partially returned on rotating. No end point was obtained. In testing duplicate samples treated exactly alike, we found the samples would not check each other. We obtained more uniform results by the method used in our laboratory.

*Collaborator No. 3.*—Considerable difficulty was experienced in completely ashing the samples when the procedure submitted was followed exactly. A char remained after all the  $\text{HNO}_3$  had boiled out. Further addition of 2 or 3 increments of 0.2 ml. of  $\text{HNO}_3$  was sufficient to clear the solution. It would appear that precautions regarding complete removal of  $\text{HNO}_3$  should be emphasized. In our hands the yellow-brown color that developed in the final solutions was suspected of being unremoved  $\text{HNO}_3$ . Color comparison was frequently found difficult particularly when the iron content was low, because of the flocculent silica precipitate.

*Collaborator No. 4.*—The addition of the cellosolve reagent causes the solution to warm up; hence the solution should be diluted to almost the desired amount and cooled immediately with ice water before addition of reagent. On standing over 15 minutes, the samples begin to fade faster than the standards. If a large number of samples are run, accuracy of reading is impaired. The time required for evaporation and digestion of sample is about 2 hours. The rapid change of color intensity, due to both heat and time, does not seem to be favorable to this method.

*Collaborator No. 5.*—The standards tended to fade quite rapidly when exposed to the strong beam of light in the photometer. The beer samples did not exhibit this tendency to fade.

*Collaborator No. 6.*—The method is undoubtedly a very sensitive one, besides being easy and rather rapid to execute. We find it superior in sensitiveness to the colorimeter methods generally used for a quick determination of iron in beer, and considerably more rapid than quantitative methods used in determining minute amounts of iron in beer samples.

TABLE 3.—*Results obtained by collaborators on two samples of beer submitted*  
(Results expressed as p.p.m. of iron.)

COLLABORATOR NO.	WET ASH-METHYL CELLOSOLVATE METHOD					OTHER METHODS		
	SAMPLE A		SAMPLE B		COLOR COMPARISON	SAMPLE A	SAMPLE B	DESCRIPTION OF METHOD USED
	IND. RESULTS	AVERAGE	IND. RESULTS	AVERAGE				
1	0.2	0.2	0.1	0.1	Nessler Tubes	1.0	0.1	HNO <sub>3</sub> and KCNS added to beer
2	1.36		0.56					Color extracted with amyl alcohol
	1.36		0.44					
3	0.96	1.23	0.64	0.55	Nessler Tubes	—	—	
	1.10		0.22		Duboscq			
	1.09	1.10	0.25	0.24	Colorimeter	0.69	0.10	Wet ashed and KCNS. Extracted with amyl alcohol
4	0.4		0.2					
5*	0.6	0.5	0.3	0.25	?	—	—	
	0.96		0.26		Neutral			Alpha alpha dipirydyl added directly to beer(3)
	1.02		0.30		Wedge	0.7	0.2	
6	0.92	0.97	0.30	0.29	Photometer	—	—	
7	1.0	1.0	0.2	0.2	Nessler Tubes			
	1.06	1.06	0.31	0.31	Photometer	0.90	0.32	Dry ashed—Direct KCNS
8	0.6	0.6	0.1	0.1	Nessler Tubes	0.3	<0.1	Dry ashed—Direct KCNS Alpha alpha dipirydyl added directly to beer (3)
9	1.60	1.60	0.50	0.50	Nessler Tubes	0.3	<0.1	
	1.12		0.28		Duboscq			
Associate	1.08	1.10	0.28	0.28	Colorimeter	—	—	
Referee	0.92		0.28		Coleman			
	0.96	0.94	0.30	0.29	Photometer			

\* This collaborator also added 0.5 p.p.m. of copper to Sample A and obtained for two determinations 1.10, 1.10.

*Collaborator No. 8.*—Colors were compared in Nessler tubes, and a color comparing Nessler tube support, with north light reflected by a milk glass plate, was used. The values obtained could not be checked by methods specifying potassium thiocyanate and alpha alpha dipyridyl. Both of these methods, however, gave lower values than checked each other.

*Collaborator No. 9.*—Some difficulty was experienced in obtaining check results on duplicate samples. It appears that the degree to which the sample was evaporated had some effect upon the results.

### DISCUSSION

The Associate Referee believes that the results of the collaborative work are not, as a whole, a true test of the method. Some of the results are in excellent agreement, while others vary widely from the average values. It will be observed that the results obtained from the collaborators that used some kind of an instrument for the final measurement of the color are much more favorable than those obtained by comparisons in Nessler tubes. Those who are interested in doing further work with this method may find it helpful to know that the maximum color absorption for photometric work lies between 475 and 490 m $\mu$ . It is quite obvious that for most accurate work, the flocculent silica precipitate in the solution should be filtered off. The fading of the final iron thiocyanate color seems to be one of the most serious objections to the method. The method of ashing seems to be quite satisfactory.

The results that have been reported on these samples by other methods emphasize the importance of a good method for the determination of iron in beer. Not only do these results differ widely from the results obtained by the wet ash-methyl cellosolve method, but they vary widely among themselves.

### RECOMMENDATIONS\*

It is recommended—

- (1) That further studies be made of this method and of other methods that may be applicable to the determination of iron in beer and that further collaborative work be done.
- (2) That work be started on the determination of copper in beer.

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\* For report by Subcommittee D and action by the Association, see *This Journal*, **24**, 63 (1941).

No report on carbon dioxide in beer was given by the associate referee.

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No report on hops was given by the associate referee.

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No report on volatile acids in wine was given by the associate referee.

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## REPORT ON SULFUR DIOXIDE IN BEER AND WINE

By L. V. TAYLOR (Research Department, American  
Can Company, Maywood, Ill.), *Associate Referee*

A previous paper by the Associate Referee, et al., *This Journal*, **20**, 610 (1937), showed that these authors obtained reliable and reproducible results for all types of wines by the Monier-Williams method for the determination of sulfur dioxide. On the basis of this work, although without collaborative data, the Associate Referee, at the 1939 meeting of the Association, recommended that the Monier-Williams method be adopted as tentative for this determination in wines, *This Journal*, **23**, 189 (1940). This recommendation was adopted by the Association, and the method now appears in *Methods of Analysis*, A.O.A.C., 1940, 170.

Prior to recommending that the method be adopted as official for wine, the Associate Referee considered it to be advisable to subject the procedure to collaborative study during the current year. Therefore samples of two types of wine, Sauterne and Port, were submitted to seven collaborators with instructions to follow the procedure and to use both gravimetric and volumetric technics in the determination of the sulfur dioxide present.

Experience had shown that the sulfur dioxide content of wine is unstable under certain conditions of storage. Consequently, efforts were made to stabilize this factor in the collaborative samples by sealing the bottles under an atmosphere of carbon dioxide prior to their distribution to the collaborators. This effort, however, proved fruitless, as evidenced by the results submitted by the collaborators (Table 1). These values range from 13 to 233 p.p.m. of sulfur dioxide in Sample W-1 (Sauterne) and from 10 to 59 p.p.m. of sulfur dioxide in Sample W-2 (Port). The highest values reported for each sample were obtained in the writer's laboratory at the time the samples were prepared for distribution. An analysis of Sample W-1 by the same collaborator approximately three weeks after its preparation showed a loss of approximately 70 p.p.m. Similar losses were noted in the results of other collaborators when they were compared with the values obtained by the Associate Referee at the time the samples were prepared. It is of interest to note that with the exception of one collabo-



rator's results, duplicate determinations checked well for a procedure of this type with either the volumetric or gravimetric method.

In general, it may be said that both the volumetric and gravimetric methods for determination of the sulfur dioxide in wine are satisfactory.

TABLE 1.—*SO<sub>2</sub> in wine*

COLLAB-ORATOR	SAMPLE W-1 (SAUTERNE)					SAMPLE W-2 (PORT)					
	DATE SAMPLE OPENED	DATE SAMPLE ANALYZED	DET'N. NO.	SO <sub>2</sub> (P.P.M.)		DATE SAMPLE OPENED	DATE SAMPLE ANALYZED	DET'N. NO.	SO <sub>2</sub> (P.P.M.)		
				VOL.	GRAY.				VOL.	GRAY.	
1	(1)	7/12	1	230	227	(1)	7/16	1	60	59	
			2	236	223			2	58	54	
	8/1	8/1	1	160	172						
			2	160	179						
	2	7/23	7/23	1	216	213	7/24	7/24	1	55	55
				2	219	215			2	55	55
3				220	215						
3	8/21	8/26	1	162	156	8/21	8/23	1	40	36	
			2	165	153			2	38	36	
4	9/3	9/3	1	169	171	9/3	9/3	1	42	37	
			2	177	175				38	35	
5	9/4	9/4	1	190	204	9/9	9/9	1	48	46	
			2	197	195			2	50	46	
			3	195	204			3	48	47	
6	9/3	9/3	1	16	76	—	—	1	10	10	
			2	22	97			2	11	10	
			3	13	—						
			4	96	50						
			5	13	27						
7	—	—	1	174	157	—	—	1	44	40	
			2	177	158			2	45	40	

(1) Initial SO<sub>2</sub> content of bulk wine used in study.

If the values reported by Collaborator 6 are excluded, the results reported by Collaborator 7 on Sample W-1 are the only ones in which there appears to be a serious discrepancy between the volumetric and gravimetric procedures. Although Analyst 6 reported that he was unable to account for the discrepancy, one of the collaborators pointed out that the use of concentrated hydrochloric acid might cause a carry over of acid fumes and thus contribute to high results by the volumetric procedure. Hence, he

suggested that it might be more desirable to use a more dilute acid in the procedure.

Satisfactory appraisal of the applicability of the Monier-Williams method to wines can not be made on the basis of the results presented here. It appears that the major problem at present is the preparation of more stable samples for collaborative study, as it is only through such studies that difficulties in the general application of the method are discovered and eliminated. The comments and suggestions of the collaborators have been helpful in analyzing the data and in suggesting future lines of approach.

The Associate Referee recommends\* that the collaborative study of sulfur dioxide in wines be continued.

Acknowledgment is made to the following collaborators:

J. B. Thompson, Jr., State Laboratories Dept., Bismarck, N. D.

L. E. Clifcorn and D. Heberlein, Continental Can Co., Chicago, Ill.

R. A. Osborn, U. S. Food and Drug Adm., Washington, D. C.

J. B. Robb, Virginia A.B.C. Board, Richmond, Va.

H. W. Edwards, Dept. of Agriculture, Lansing, Mich.

C. G. Ryberg, American Can Company, Maywood, Ill.

Stephen Laufer and Julius Siebenberg, Schwarz Laboratories, New York City.

## REPORT ON DENATURANTS (METHANOL) IN DISTILLED SPIRITS

By G. F. BEYER (Alcohol Tax Unit, Treasury Department,  
Washington, D. C.), *Associate Referee*

At the last meeting of the Association a method for the quantitative determination of methanol in distilled spirits was submitted for tentative adoption. The Committee on Recommendations decided that it was necessary to show satisfactory results from collaborative work before the method could be adopted as official. However, before any further work in this direction was done, an effort was made to shorten the time of distillation specified in the tentative method. Therefore, a number of distillations were made as outlined in the method, and another series of distillations was made by taking off the distillate at the rate of about 22 drops per minute, instead of 1.2 ml. every 15 minutes. An analysis of these distillates showed that there was no difference in the methanol content.

Further work was also done with the still described for this purpose, to determine the alcoholic strength of the distillate when a sample is distilled in the manner referred to previously. Several determinations showed that this distillate contained 96 per cent of alcohol by volume.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 63 (1941).

Since the work in the Washington laboratory showed no difference in the two methods of distillation, a number of samples containing 0.1 per cent of methanol by volume in approximately 50 per cent ethyl alcohol was sent to nine field laboratories of the Bureau of Internal Revenue, and to three others.

A report from the Bureau's laboratories shows that one analyst found 0.1 per cent; one found 0.095 per cent; one found 0.094 per cent; two found 0.09 per cent; and two found slightly more than 0.06 per cent. One analyst was somewhat indefinite, reporting not more than 0.1 per cent. No other reports were received.

No explanation can be given for the two low results of 0.06 per cent, unless no correction was made for the dilution of the distillate. This reason is presented because two laboratories found that the color obtained matched the 0.06 per cent standard and that when the dilution of the distillate was taken into consideration approximately the correct result was obtained.

All the laboratories reported that there was no difference in the color obtained from the two methods of distillation and that the continuous method was preferable. Since these reports are most encouraging, a further effort will be made to obtain other collaborators so that as a result of satisfactory data the method, with the proposed change in the manner of distillation, may be adopted as official.

## REPORT ON WHISKEY AND RUM

### TANNINS IN POTABLE SPIRITS

By PETER VALAER (Alcohol Tax Unit, Treasury Department,  
Washington, D. C.), *Associate Referee*

One important phase of the chemistry of potable spirits that apparently has been underestimated and no doubt overlooked to some degree is tannin content. It appears that for the interpretation of analyses a short, simple, reproducible method for total tannins in potable spirits is almost indispensable. Such a method must be fairly accurate and so readily usable that it would be practicable for all spirit chemists wishing to measure their products by a similar yardstick. Naturally the analyst would not expect to find tannins in ordinary potable spirits that have had no contact with wood, but it is quite likely that a knowledge of tannin content would give valuable additional knowledge of the character of the total solids found on analysis.

While tannins in general do not appear to be definitely organized bodies, but rather to be complex substances of uncertain composition, the problem is simplified to some extent in the case of potable spirits in that the

analyst is only concerned with the tannin material extracted by the spirits during storage from seasoned white oak wood, which may be plain, charred, or reused. The whiskey that has aged the longest in newly charred, white oak containers will have the largest proportion of tannin extracted from the wood, and the tannin content will increase gradually and become more concentrated during aging. Whiskey (and other potable spirits) will extract more tannin during the first six months of storage, just as it has been shown that most of the acids, esters, color, and solids are developed and extracted during this earlier stage.<sup>1</sup> Tannins are quite soluble in water and dilute alcohol and are leached out of the white oak wood along with the soluble coloring matter; they are colorless but are usually associated with brown coloring substances. In the fresh material they are in solution, but on drying they impregnate the tissues or form brown deposits. Scotch and other whiskies that are aged in reused or in uncharred barrels will show relatively smaller amounts of tannin. This situation, however, may be made more complex and the time of storage more difficult to estimate when spirits are aged artificially by quick-age processes, such as abnormally higher heat and the use of white oak chips. The amounts of tannin found in such quick-aged products will be out of proportion with the usual ingredients and will show an unusual color-solids ratio. During aging a proportionately larger amount of color and tannin will be evident before the acids are obtained by oxidation or esters are formed by esterification.

In the publication of analyses of potable spirits the tannin content, as such, is usually absent, although it is included in "total solids" or "extracts" content. It is believed by many analysts that the tannin determination is quite likely to have an important bearing on the aging history of any spirit beverage. It is also likely that less is known of the true nature of the tannin substance than of any other ingredient so far found in aged spirits.

A chemical method for the determination of tannin in potable spirits seems never to have been considered by the A.O.A.C., but the Associate Referee found several processes that are being used at the present time that may be considered. The present A.O.A.C. method for the determination of tannin in wine is usually applied to aged spirits. However, to chemists who have used it and compared it with other methods, it seems too long and detailed for the analysis of simpler substances like potable spirits. The method for the determination of tannin in wine appeared in 1907, in U. S. Div. Chem. Bulletin 107, which was the early *Methods of Analyses*, A.O.A.C., and it has been continued in the 1940 Edition, 169, 31, which makes it official for 38 years. This may indicate that the method has proved satisfactory all these years, or it may be a simple case of neglect.

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<sup>1</sup> Valaer, Peter, and Frazier, W. H., *Ind. Eng. Chem.*, 28, 92 (1936).

The present A.O.A.C. method was originally taken from Neubauer.<sup>2</sup> This method is used by practically all wine chemists that determine tannin, and it is also used by the laboratories of at least two of the largest distilling companies. A simpler method of Folin and Denis<sup>3</sup> is based on the reaction of the Folin-Denis reagent (sodium tungstate phosphomolybdic acid and phosphoric acid, with sodium carbonate) with the tannins producing a deep blue color, which is read colorimetrically in Nessler tubes, or more accurately in a photometer. The latter process is now extensively used for the determination of tannins in spirits. The Folin-Denis method and the A.O.A.C. method previously mentioned gave about the same results on the same samples of whiskey, which is remarkable when one considers the great difference in the character of the two methods. As may be expected, the A.O.A.C. method generally gives slightly higher results. Briefly, the A.O.A.C. method for tannin in wine is the titration of the de-alcoholized sample with standard potassium permanganate solution, with indigo solution used as an indicator. As the potassium permanganate solution reacts with the coloring matter as well as with the tannin, the value obtained is known as "tannin plus coloring matter." Since reducing matter other than tannin is present, it is necessary to make a blank titration. This is done after decoloration with carbon, which removes the reducing constituents from the wine. It has been found that without further treatment the high-grade commercial carbons, like Darko, give the same results as does the acid- and water-washed boneblack. This carbon is also much more convenient to use.

The tannin in wine is quite generally considered to be exclusively of the catechol type. The hide powder method for tannin in tanning material has been successfully applied to wine, but it appears more complicated and time-consuming than the more frequently used methods mentioned above.

Another method considered was that of Astruc and Castel,<sup>4</sup> which is also colorimetric, as is the Broeman method (unpublished), the former specifying 1 per cent ferric chloride with 20 per cent sodium acetate solutions, the latter using ferric chloride (5 per cent) with caramel solution in the standards. With the latter method accurate comparisons are difficult because ferric chloride produces different colors in the samples and in the standards to be used for comparison.

Tannic acid, gallo-tannic acid, tannins, and di-gallic acid are terms used interchangeably for the same substance, and they are given the formula  $H \cdot C_{14}H_9O_9$  or  $C_{14}H_{10}O_9$ . It is this well-known chemical that has appeared in the U. S. Pharmacopoeia for the past 50 years with its specifications practically unchanged from the original copy. It is obtained principally

<sup>2</sup> *Ann. Oenologie*, 2, 1 (1872).

<sup>3</sup> *J. Biol. Chem.*, 12, 239 (1912).

<sup>4</sup> *Ann. fals.*, 25, 477 (1932).

from the nut galls, but regardless of its source it is official if it meets the U.S.P. standards. The chemically pure variety that is prepared by manufacturing chemists for medicinal and chemical purposes is used in the laboratory for a standard of comparison.

The tannin found in all grape wines has been extracted from the stems, the pips, and the skins. Because the wine is in contact with the skins for a longer period, the tannins are much more abundant in red wines than in white wines, and the astringency of red wine is due principally to its tannin content. Tannin is also found in all other fruit wines, such as peach, apple, cherry, and pear, as well as in all berry wines, such as raspberry, loganberry, and blackberry. Blackberry secretes much more tannin than does any other wine, a fact that accounts for its medicinal uses. Dekker<sup>5</sup> describes three types of true tannins somewhat similar in their reactions, those that are derivatives of gallic acid, ellagic acid, and catechol, respectively.

Those wishing to determine tannin in wine or potable spirits usually employ the A.O.A.C. method for tannins in wine, but the following shorter procedure is also used in many winery laboratories:

Measure 25 ml. of filtrate prepared as directed in the A.O.A.C. method into a large porcelain evaporating dish. Add 20 ml. (carefully measured) of indigo solution and 1000 ml. of distilled water. Titrate the solution with 0.1 *N* permanganate, stirring thoroughly until the blue color of the solution begins to change to green, then add the permanganate more slowly until the color changes to greenish yellow. Allow the liquid to stand 2-3 minutes and then add standard permanganate dropwise with thorough mixing until a bright golden yellow color is obtained.

The A.O.A.C. method is based on the fact first worked out by Lowenthal<sup>6</sup> that tannin is oxidized in acid solution by permanganate. The oxidation, however, proceeds slowly and the end point is indefinite. By the addition of a quantity of indigo solution (sodium indigotin disulfonate), which must be pure, otherwise the end point is indefinite, the oxidation of the tannin is controlled and the end point can be recognized by the change in color.

In addition to alcohol wine contains certain nonvolatile substances that are capable of reducing permanganate. The alcohol is removed by evaporation and the tannin and coloring matter are held back by the boneblack, while the other oxidizable substances are determined in the second titration. Different batches of purchased sodium indigotin have varied to some extent, but if FD & C Blue No. 2 (1180 indigotin) certified is obtained the results by this method should be more consistent.

In order to decide between the two best methods in use at present for the quantitative determination of tannin or to discover a more suitable one, 20 sets of samples were sent to as many collaborators. Each set con-

<sup>5</sup> Gerber, 54, 113, 122, 130, 150, 155 (1928).

<sup>6</sup> *Z. Anal. Chem.*, 16, 33 (1877).

sisted of one sample of whiskey aged in a charred barrel and one sample of brandy aged in an uncharred barrel. It was requested that the collaborators determine tannin by the present A.O.A.C. method for wine (*loc. cit.*) and also by the proposed method, which follows:

### TANNIN

#### REAGENTS

(a) *Folin-Denis solution*.—To 750 ml. of distilled water add 100 grams of sodium tungstate, 20 grams of phosphomolybdic acid, and 50 ml. of 85% phosphoric acid. Reflux for 2 hours, cool, and dilute to 1 liter.

(b) *Saturated sodium carbonate solution*.—For each 100 ml. of water at 70°–80° C. add 35 grams of anhydrous  $\text{Na}_2\text{CO}_3$ , dissolve, and allow to cool overnight. Feed the supersaturated solution with a crystal of  $\text{Na}_2\text{CO}_3$ , and after crystallization filter through glass wool.

(c) *Standard tannic acid solution*.—Dissolve exactly 100 mg. of tannic acid in 1 liter of distilled water. Prepare a fresh solution for each determination.

#### DETERMINATION

Place 0.25–1.00 ml. of whiskey in a Nessler tube containing approximately 90 ml. of water. Add 1.0 ml. of the Folin-Denis reagent and make up to mark with water. Then add 5.0 ml. of the  $\text{Na}_2\text{CO}_3$  solution and shake well. After 10–15 minutes, compare the blue color developed with standards made in the same way at the same time containing 0.0, 0.4, 0.6, 0.8, 1.0, 1.6, 2.0, and 2.4 ml of the standard tannic acid solution.

The collaborators were requested to make suggestions for improving both methods and to give their opinions as to which method would be more suitable for the determination of tannin in potable spirits. They were also requested to suggest a third method. The valuable comments and the suggestions made by the collaborators, as well as the results of their analyses, follow.

#### *Results on tannin obtained by collaborators* (Grams per 100 liters.)

COLLABORATOR	SAMPLE NO. 1—WHISKEY		SAMPLE NO. 2—BRANDY	
	PROPOSED METHOD	A.O.A.C. METHOD	PROPOSED METHOD	A.O.A.C. METHOD
Burrit, Loren	35	37	25	41
Washington	35		25	
	35		25	
			25	
Nealon, E. J.	30	42	22	30
Detroit				
Hamill, Geo. K.	39	41	25	28
Washington				
Fonner, J. F.	30	33	24	25
Chicago				
Dale, Lloyd		36		27
Kansas City	34	31	22	22
		32		23

*Results on tannin obtained by collaborators—Continued*

COLLABORATOR	SAMPLE NO. 1—WHISKEY		SAMPLE NO. 2—BRANDY	
	PROPOSED METHOD	A.O.A.C. METHOD	PROPOSED METHOD	A.O.A.C. METHOD
Mallory, Geo.	29	31		19
Los Angeles	31	36	18	24
Mahaffie, O. B.	32	47*	20	42
Pittsburgh				
Mottern, A. J.	32	42	20	18*
Philadelphia	32	32	20	23
	30		20	
	32			
Quillen, J. W.	36	42	20	30
Baltimore				
Morawski, A. L.	28	40	20	24
Boston				
Holman, S. W.	24	42	16*	33*
Atlanta				
Love, R. F.	30	36	19	26
San Francisco				
Liebmann & Rosenblatt	35		22	
(Schenley) New York	34		22	
Carson, C. T.	30		20	
(Frankfort) Baltimore				
Dr. C. S. Boruff	28		24	
(Hiram Walker)				
Peoria				
A. Herman	23		19	
J. E. Seagram				
Louisville				
Smith & Robb	32		23	
(Virginia A.B.C. Board)	(11 determi-		(10 determi-	
Richmond	nations)		nations)	
Sipherd, I. R.	35		22	
National Distillers	(Av. 7 labs.		(Av. 7 labs.	
New York	Nat. Distillers)		Nat. Distillers)	
Ladd and Drum	35		23	
Bismarck	36		24	
Romig and Milos	28	33	22	23
New York	32	35	22	24
	29	36	22	
	29			
	29			
Wilson, J. B.	36	32	20	16*
Food and Drug Adm.	34	33	20	17*
Washington			19	
Valaer, Peter	35	37	25	25
Washington	35	37	25	27
		39		
Burrit, Loren	40*	37	25	23



*Results on tannin obtained by collaborators—Continued*

COLLABORATOR	SAMPLE NO. 1—WHISKY		SAMPLE NO. 2—BRANDY	
	PROPOSED METHOD	A.O.A.C. METHOD	PROPOSED METHOD	A.O.A.C. METHOD
Washington	35	37 37 39	25 25	25
Total	1224	1032	815	586
Average	32.2	36.8	22.0	24.4
Maximum	39	42	25	30
Minimum	23	31	18	19

\* Averaged but not accepted as maximum or minimum because they are judged abnormally low or high.

## COMMENTS AND SUGGESTIONS

*J. F. Fonner.*—I prefer the proposed method because it is much shorter and easier. This method gives an estimation of the amount of tannin present and that is all that is required in the ordinary analyses of spirits. This method can be made slightly more accurate by decreasing the difference between the standards, such as 0.2 ml. variation, after ascertaining the approximate amount of tannin in the spirits.

*George Mallory.*—The proposed method is exceedingly rapid for the determination of tannin in potable spirits and should be more reliable than the present A.O.A.C. official method (in wine), which necessarily includes the oxidation of coloring material also.

*A. J. Mottern.*—The Folin-Denis method is easy of application, fair, and rapid, and it gives results that may be readily duplicated. The A.O.A.C. procedure is longer, requires more sample solution and more manipulation, and the end point is not quite so sharp as might be desired, so that great care is necessary in order to obtain consistent results.

*A. L. Morawski.*—I found the proposed method to be shorter and experienced no difficulty in checking results several times during two weeks. The A.O.A.C. method is long, particularly the purifying of the animal charcoal of the chlorides. Untreated Darco seemed to work as well as the treated animal charcoal.

*S. W. Holman.*—It is obvious from our analyses that these methods do not correspond, and for that reason we have checked and rechecked, but there is still a wide variation. We believe that the A.O.A.C. method is preferable because of the sharp end point.

*E. J. Nealon.*—The Folin-Denis reagent method for the determination of tannin is much simpler and quicker than the A.O.A.C. method. Although it gives consistent results on check determinations, the results are considerably lower than those obtained with the A.O.A.C. method. The A.O.A.C. method shows slight variances on check determinations. It is the opinion of the Detroit Laboratory that the A.O.A.C. method actually determines the organic matter and the tannin, which are removed by boneblack treatment, and consequently higher results could be expected. Based on this theory, our opinion would be that the method specifying the Folin-Denis reagent is more accurate than the A.O.A.C. method.

*Lloyd Dale.*—The procedure with the Folin-Denis reagent appears to be at least as accurate as the A.O.A.C. method and gives more readily reproducible results.

*Ladd and Dunn.*—A photometer gave very satisfactory results, and it eliminated the preparation of fresh standard tannic acid solution for each determination. Samples of 0.5 ml. and 25 ml. were more satisfactory than larger samples, which gave too intense color for comparison. Standards containing more than 2 ml. of standard tannic acid solution were too intense for comparison; standards in excess of this concentration showed a less marked color difference. This fact is illustrated by the curve plotted from the photometer readings. The smaller samples showed slightly more than the larger ones.

*J. W. Quillen.*—From a manipulation standpoint the colorimetric method is preferable to the A.O.A.C. method, as the determinations can be made quite rapidly by the colorimetric method, once the solutions are made up.

*Smith and Robb.*—The low-form Nessler tubes gave the best checks; with these tubes and 0.5 ml. or less of the unknown consistently duplicated results were obtained.

*A. Herman.*—We found it more convenient to add  $\text{Na}_2\text{CO}_3$ , and then to make to the mark. There is a possibility that all tannic acid samples may not be the same. Our standard was weighed up on a dry basis so that the standard solution contained 100 mg. of dry tannic acid per 1000 ml.

*R. F. Love.*—The C.P. tannin used for standards in the colorimetric method was found to have 6.6 per cent moisture, and the standard solutions were calculated on the basis of dried tannin.

In the A.O.A.C. method a precipitate is formed on the dealcoholization, which does not dissolve on dilution to the original volume. This precipitate was not filtered off, but was included in the solution analyzed. No doubt removal of precipitate would change the result of the analysis. The colorimetric method seems to be satisfactory. It requires less time and manipulation than the A.O.A.C. method, and the tannin is determined without including other coloring matter. Some trouble was experienced in preparing a saturated solution of sodium carbonate by the method given; 35 grams of  $\text{Na}_2\text{CO}_3$  does not allow sufficient excess when the room temperature is above 75° F.

*Romig and Milos.*—In the A.O.A.C. method two separate portions of the same sample are titrated with  $\text{KMnO}_4$ . During this titration the solution changes from blue to green, to golden yellow, the end point. At best, this end point can be determined to within 0.1 ml. of standard permanganate. Since 0.1 ml. of permanganate solution is equivalent to 1.7 grams of tannins per 100 liters, the results obtained by this method may be in error by 3.4 grams per 100 liters.

The colorimetric method consumes less time than the A.O.A.C. method of analysis. In both the distilled spirits the tint of blue color that developed was different from that produced by the standard tannic acid solutions. Exact comparisons of colors within narrow limits are always difficult, and the variance in tint increases this difficulty. When the depth of color of the unknown varied between two standards, an intermediate standard was prepared for comparison purposes. Also a Schreiner colorimeter was employed. It gave about the same results as those obtained with intermediate standards. Assuming the color produced is due to the presence of tannins, I think that this method is accurate to within 4 grams per 100 liters.

In view of the satisfactory results obtained by most of the chemists in this collaboration, it is recommended\* that this method be adopted as tentative.

\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 63 (1941).

No report on detection of adulteration of distilled spirits was given by the associate referee.

No report on wood alcohol in distilled spirits was given by the associate referee.

## REPORT ON CORDIALS AND LIQUEURS

By JOHN B. WILSON (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

No collaborative work was done this year since it was the opinion of the Associate Referee that the results obtained in previous years warranted the official adoption of the methods for benzaldehyde, volatile esters, gamma-undecalactone (qualitative), and total solids from the refractive index of the dealcoholized sample.

### RECOMMENDATIONS\*

It is recommended—

(1) That the method for the determination of benzaldehyde, *Methods of Analysis*, A.O.A.C., 1940, 184, 63, be adopted as official (final action).

(2) That the method for the determination of volatile esters in cordials, *Ibid.*, 183, 60, be adopted as official (final action).

(3) That the method for gamma-undecalactone (qualitative), *Ibid.*, 183, 61, be adopted as official (final action).

(4) That the method for the determination of total solids from refractive index of dealcoholized sample, *Ibid.*, 181, 44(c), be adopted as official (final action).

## REPORT ON SOILS AND LIMING MATERIALS

By W. H. MACINTIRE (The University of Tennessee Agricultural  
Experiment Station, Knoxville, Tenn.), *Referee*

The efforts of the Referee have been chiefly of an advisory and suggestive nature, except for certain studies conducted with J. W. Hammond as to the determination of the fluorine content of incinerates of unusually high silica content.

The present method for the distillation of fluorine from CaO<sub>2</sub>-treated soils, and MgO<sub>2</sub>-plant incinerates, has proved satisfactory, except for certain soil samples and for certain crops from soils limed with calcium silicate. Unsatisfactory results were obtained in attempts to effect complete separation of fluoride solutes from silica through precipitation with zinc oxide, as preliminary to perchloric acid distillation. Two-step dis-

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 63 (1941).

tillation—with sulfuric acid and redistillation of the concentrate with perchloric acid—gave fairly satisfactory recovery from  $MgO_2$ -incinerated material. A variation introduced was the heating of the suspension-solution of incinerate with concentrated sulfuric acid to fumes of sulfur trioxide to effect dehydration of silica, the small volume of distillate being trapped with sodium hydroxide and returned to the flask for redistillation. Because of the disadvantages of time, augmented blanks, and attendant indefiniteness of end point of titration, as well as difficulty in obtaining fluoride-free peroxides of calcium and magnesium, further attempts were made to obviate the difficulty of interference of high silica incinerates.

Analytical charges were treated with 10 ml. of a 5 per cent aqueous suspension of fluorine-free calcium hydroxide, dried at  $105^\circ C.$ , and then ignited to whiteness at  $550^\circ C.$  in an electric furnace. The incinerate was then transferred to the usual distillation apparatus; 0.1 gram of either sulfate or perchlorate of silver was introduced; distillation was conducted with perchloric acid, at  $135^\circ C.$ ; and the distillate was titrated with 0.01 *N* thorium nitrate. The requisite volume of distillate in relation to variable material and analytical charge should be determined empirically.

It is recommended\*—

- (1) That the technic suggested be subjected to collaborative study.
- (2) That the work of the Associate Referee on H-ion Concentration of Soils of Arid, Semi-arid and Humid Regions be continued.
- (3) That Recommendations 1 and 2 of the Associate Referee on Liming Materials be approved.
- (4) That the work on boron be continued.

## REPORT ON H-ION CONCENTRATION OF SOILS OF ARID AND SEMI-ARID REGIONS

By W. T. MCGEORGE (Arizona Agricultural Experiment Station,  
Tucson, Arizona), *Associate Referee*

Last year the report presented was in the nature of a statistical analysis of data submitted by a number of collaborators. At the same time a method was proposed for *pH* determination in semi-arid soils. This method was based on the work of the collaborators and subsequent investigations conducted by the Associate Referee.

During the past year ten collaborators assisted in additional cooperative work, which is presented herewith (see following paper). This report also presents further evidence that the method proposed last year is the most suitable one for semi-arid soils.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 48 (1941).

DETERMINATION OF *pH* VALUE FOR ALKALI SOILS

By W. T. McGEORGE and W. P. MARTIN (Agricultural  
Experiment Station, Tucson, Ariz.)

The attainment of greater accuracy from improved methods and apparatus available for determining the *pH* value of soils has added greater significance to such data. In spite of these improvements, however, wide variations in analytical procedure and the *pH* values thus obtained continue.

The results obtained by a cooperative study of the *pH* determination in semi-arid soils for 1939, *This Journal*, 23, 205 (1940), were not considered satisfactory. The disagreement among the eight analysts who collaborated was considerably greater than the limits of error that had been selected as attainable, namely 0.2 *pH* units. In view of this, the work was continued for another year, and determinations were made both at low moisture content, approaching field moisture content, and at a 1:5 soil-water ratio, which has been the ratio most often used in past years.

Owing to the hydrolysis of the potentially acid and alkaline compounds in the soil, when in contact with water, the *pH* value increases with increase in soil-water ratio and reaches a maximum value with boiled distilled water at approximately a ratio of 1:10. The question then arises as to the value or usefulness of these different factors. The agronomist and the farmer are probably most interested in the *pH* value of the soil under field conditions, approximately the moisture equivalent. The physiologist and the soil technologist are interested in the fundamental nature of *pH* and the hydroxyl- and hydrogen-ion concentrations of the soil solution under variable conditions. Obviously, then, if one is interested in the hydrolytic properties, the buffer capacity, and the acid or alkali compounds responsible for the *pH* value, the *pH* determination should be made at several soil-water ratios. By means of the glass electrode it is possible to determine the *pH* of a soil that will supply sufficient moisture to make a continuous moisture film contact between the glass and calomel electrodes.

In general, it seems that a *pH* determination made on the soil at field moisture content should have the greatest practical value. However, the data obtained from the collaborative work of 1939 showed very poor agreement among analysts for this determination. There may have been two reasons for this—first, the lack of experience with the technic of making the determination by this new procedure, and, second, an error arising from incomplete mixing of such a small quantity of water with the soil and establishing contact and equilibrium between the electrodes and the soil. Preceding the collaborative work for 1940 further investigations were made on the possible sources of error.

## METHOD OF ADDING WATER TO THE SOIL

The pH determination at low moisture content can be made by several methods: First, by taking the equipment into the field where the electrodes can be pressed into the soil, if wet enough, otherwise using a small quantity of water to wet the soil to the point where the electrodes may be pressed gently into it; second, by adding sufficient water to the air-dried soil, in a beaker, to bring the soil to approximately the moisture equivalent, stirring thoroughly, and pressing the electrodes into it; third, by placing a Gooch crucible containing soil in a reservoir of water and wetting the soil by capillarity as in the Bouyoucus suction method for determining the moisture equivalent. In view of the limitations in collaborative work of this type, this study was confined to the second and third methods.

The simplicity of the capillary method has certain advantages, but the experiments showed that it is not workable on all soil types. Highly organic soils, especially peat and muck, and some black alkali soils, could not be wet by capillarity. Also the pH values obtained when the soils were wet by capillarity were in some cases slightly lower than the values obtained by mixing the water with the soil in a beaker. This is shown by the data in Table 1.

TABLE 1.—*pH values at water holding capacity by beaker and capillary methods*

SOIL NO.	pH AT W.H.C. CAPILLARITY	pH AT W.H.C. CAPILLARITY*	pH BY BEAKER METHOD
1	7.40	7.40	7.50
2	9.70	9.65	9.95
3	9.50	9.65	9.80
4	7.60	7.55	7.70
5	7.65	7.70	7.75
6	7.45	7.50	7.45
7	7.45	7.65	7.55
8	5.30	5.35	5.30

\* Saturated by capillarity in desiccator at reduced pressure.

The pH values given in the first column were obtained by keeping the Gooch crucibles containing the soil in a reservoir of distilled water until the soil was completely wetted and then allowing them to drain free of excess water. Because the results thus obtained were lower than those obtained by the beaker method, in which the soil and water were thoroughly stirred, the same procedure was repeated by obtaining capillary saturation in a vacuum desiccator at reduced pressure. These data are given in Column 2. It will be noted that these pH values are also lower than those obtained by the beaker method.

When the soils saturated by capillarity were allowed to stand 12 hours, the pH values were in agreement with those obtained by the beaker method, showing that some time is required for the soil and water to

reach equilibrium. However, this modification introduces an additional error in the case of black alkali soils, for if such soils are not protected from the air during this period the absorption of carbon dioxide will lower the *pH* value. Therefore, the conclusion from a study of the capillary method is that except for black alkali soils and highly organic soils it will give *pH* values in close agreement with the values obtained by the beaker method if the soils are allowed to stand 12 hours after saturation.

#### INFLUENCE OF MOISTURE CONTENT BELOW WATER HOLDING CAPACITY

Since the beaker method appeared to be best suited for all conditions and soil types the next subject to be studied was that of the influence of slight variations in moisture content on the *pH* value. Obviously, if it were necessary to determine the moisture equivalent or water holding capacity of the soil before making a *pH* determination, its value in routine soil analysis would be limited. A group of nine soils was selected, and *pH* determinations were made at 1:5 soil-water ratio, the water holding capacity, and the moisture equivalent. These data are given in Table 2.

TABLE 2.—*pH* value at 1:5, water holding capacity, and moisture equivalent

SOIL NO.	<i>pH</i> 1:5	<i>pH</i> w.h.c.	<i>pH</i> m.e.
1	8.25	7.50	7.50
2	10.35	10.00	9.90
3	10.35	9.85	9.80
4	8.15	7.50	7.35
5	10.40	9.65	9.60
6	8.45	7.85	7.75
7	7.90	7.50	7.50
8	8.10	7.55	7.50
9	5.65	5.35	5.30

These data show that the *pH* value at the moisture equivalent is in very close agreement with that at the water holding capacity and clearly within the limits of experimental error for soils. It is apparent, therefore, that no accuracy is required in the addition of water in the beaker method as long as the addition does not exceed the water holding capacity. The *pH* value thus obtained will closely approximate the *pH* value of the soil under field conditions.

#### COLLABORATIVE RESULTS

After the investigations cited had been finished, 9 soils were selected and sent out to 13 collaborators in 11 Western States. Ten collaborators reported results. The samples, gathered from 6 of the Western States, represented acid, nearly neutral, slightly alkaline, and strongly alkaline soils. Instructions sent to the collaborators were as follows:

1. Weigh 20 grams of soil into a 50 ml. beaker. Add the designated quantity of water for each soil (approximately the moisture equivalent):

SOIL NO.	WATER ml.	SOIL NO.	WATER ml.
1	6.5	6	7
2	6.5	7	9
3	4.0	8	9
4	3.5	9	11
5	6.5		

Mix thoroughly with a stirring rod for about 1 minute. Press the electrodes into the soil paste and record pH readings. Take several readings on each soil immediately after the soil and water have been mixed by removing the electrodes from the paste and reinserting them.

2. Weigh 10 grams of soil into a wide-mouthed bottle (100 ml. vol.). Add 50 ml. of boiled distilled water, shake well by hand, let stand 15 minutes, shake again, and determine pH in this 1:5 suspension. (Since the supernatant liquid often has a different pH from that of the soil suspension care should be taken to make the reading in a well-stirred mixture.)

Analyses were obtained from the following collaborators.

- A. J. E. Fletcher, Soil Conservation Service, Tucson, Ariz.
- B. W. T. McGeorge.
- C. A. R. C. Haas, Citrus Experiment Station, Riverside, Calif.
- D. J. B. Page, University of California, Berkeley, Calif.
- E. R. F. Reitemier, Regional Salinity Laboratory, Riverside, Calif.
- F. R. Gardner, Agricultural Experiment Station, Ft. Collins, Colo.
- G. D. Peterson, Agricultural Experiment Station, Logan, Utah.
- H. L. T. Kardos, Agricultural Experiment Station, Pullman, Wash.
- I. T. J. Dunnewald, Agricultural Experiment Station, Laramie, Wyo.
- J. A. B. Caster, University of Arizona, Tucson, Ariz.
- K. W. L. Powers, Agricultural Experiment Station, Corvallis, Ore.

The pH determinations for the nine soils tested, as reported by the different analysts, are given in Table 3, together with the pH range, the mean pH, and the standard deviation. In addition, wherever the pH value was outside the range of the mean plus or minus the standard deviation, it was starred in order to emphasize the results showing the greatest distance from the mean. It will be noted that these stars are fairly well distributed among the results reported by the different analysts except for data submitted by Analyst I on the soils at field moisture content. All but one of these results are outside the critical range. Closer examination of these data shows that the pH values are neither consistently above nor below the average value. Such variability can not therefore be attributed to a constant source of error in the pH determinations.

In spite of lack of consistency with one set of data out of the eight, the data submitted by the different analysts are comparatively uniform. The average pH range for the samples run at both field moisture content and on 1:5 soil-water suspension is 0.44 pH units. When Analyst I's results



TABLE 3.—*pH* values for different western soils as determined by analysts from several Western States, using the glass electrode standardized against the same buffer solution

SAMPLE NUMBER	ANALYSTS										pH RANGE	MEAN pH	STANDARD DEVIATION
	A	B	C	D	E	F	G	H	I	K			
Measurements made on soils at "Field Moisture Content"													
9 (1)	5.40	5.35	5.49	5.15*	5.46	5.21*	—	—	5.60*	—	0.45	5.380	0.158
1 (2)	7.46	7.50	7.56*	7.30	7.43	7.41	7.47	7.50	7.05*	7.43	0.51	7.411	0.180
7 (3)	7.58	7.50	7.50	7.56	7.54	7.39*	7.48	7.43	7.90*	7.44	0.51	7.532	0.153
4 (4)	7.52	7.50	7.67	7.65	7.33*	7.55	7.48	7.63	7.82*	7.69	0.49	7.584	0.146
8 (5)	7.54	7.55	7.71	7.43*	7.67	7.51	7.62	7.63	7.92*	7.46*	0.49	7.604	0.141
6 (6)	7.75	7.75	7.87	7.73	7.80	7.75	7.75	7.74	8.00*	7.75	0.27	7.789	0.104
5 (7)	9.64	9.65	9.66	9.37*	9.54	9.90*	9.84*	9.41*	9.45*	9.79*	0.53	9.625	0.173
3 (8)	9.78	9.80	9.89	9.66*	9.91	9.98	10.02*	9.80	9.83	9.68*	0.36	9.835	0.121
2 (9)	9.85	9.90	10.05	9.90	10.04	10.12*	9.99	9.88	9.80*	9.91	0.32	9.944	0.095
Measurements made on 1:5 "Soil-Water Suspension"													
9 (1)	5.70	5.65	5.78*	5.60	5.69	5.08*	—	—	5.75	—	0.70	5.607	0.242
7 (3)	7.76	7.90	7.99	7.83	7.90	7.89	7.99	7.91	7.91	7.97	0.23	7.905	0.072
8 (5)	7.98	8.10	8.10	8.09	8.13	8.00	8.13	8.07	8.08	8.15	0.17	8.083	0.052
4 (4)	8.10	8.15	8.32*	8.14	7.79*	8.21	8.28*	8.00	8.14	7.88	0.53	8.101	0.158
1 (2)	8.30	8.25	8.42	7.83*	8.21	8.40	8.48*	8.30	8.44*	8.38*	0.65	8.301	0.202
6 (6)	8.35	8.45	8.40	8.36	8.19*	8.44	8.45	8.39	8.45	8.45	0.26	8.397	0.084
3 (8)	10.15	10.35*	10.34	10.23	10.37	10.62*	10.44	10.32	10.34	10.44	0.47	10.360	0.136
5 (7)	10.22	10.40*	10.36	10.20*	10.50	10.62*	10.47	10.34	10.44	10.61*	0.42	10.416	0.152
2 (9)	10.30	10.35*	10.43	10.25*	10.50	10.79*	10.53	10.40	10.47	10.60*	0.54	10.462	0.158

\* Refers to pH values outside the range: Mean  $\pm$  (Av. St. Dev. = 0.14).

are eliminated, the values are 0.25 and 0.44 pH units, respectively. This is a decided improvement over the data submitted last year when the average pH range was 1.06 at field moisture content and 0.86 at a 1:5 soil-water ratio.

The average standard deviation for the different samples run at the two moisture contents is identical and equal to 0.14 pH units. This is also a distinct improvement over the 0.19 deviation of the samples submitted last year. When Analyst I's data for field moisture content are eliminated, the average standard deviation is but 0.11 pH units.

As a basis for judging whether or not the variation among analysts is large or small, it is obviously necessary to know what variation a single analyst would obtain on several aliquots of each sample. For this purpose, three of the samples were chosen to represent a cross-section of the reactions found in the original nine samples; namely, Samples 5, 6, and 7, whose mean pH values at field-moisture content were 9.61, 7.79, and 7.54, respectively. Sixteen aliquots of each sample were prepared and given to Analysts A and J for analysis. These analysts assigned numbers to the entire 48 samples and randomized them. The pH determinations were then made at random, the same procedure being used as that employed by the other analysts. The results obtained are given in Table 4.

It may be noted that Analyst A's results are less variable than Analyst J's and that the mean pH values do not agree, in each instance Analyst A reporting the higher mean pH value. However, as expected, both analysts report results that are less variable than those found by the several analysts. The average results for the three samples tested, together with the variation found in the results reported by the several analysts using a single aliquot of each sample, are as follows:

<i>Field moisture</i>	<i>Range</i>	<i>Standard Deviation</i>
Analyst A . . . . .	0.087	0.035
Analyst J . . . . .	0.147	0.059
Analysts A to H+K . . . . .	0.287	0.098
<i>1:5 soil-water ratio</i>		
Analyst A . . . . .	0.057	0.021
Analyst J . . . . .	0.140	0.047
Analysts A to H+K . . . . .	0.303	0.102

The standard deviation of the results reported by Analyst J is roughly half as great as that for Analysts A to H, and Analyst A reported results that are even less variable than those reported by Analyst J.

While it is evident that the variation among analysts is greater than that within analysts, or more accurately between aliquots of a single soil sample as determined by a single analyst, the difference between the two is roughly but 0.05 pH units. This is less than the variation between field

TABLE 4.—Variations in reaction between aliquots of the same soil sample as determined by individual analysts

SAMPLE NO.	ANALYST	ALIQOTS										pH RANGE	MEAN pH	STANDARD DEVIATION
Measurements made on soils at "Field-Moisture Content"														
3	A	7.43	7.43	7.43	7.45	7.45	7.48	7.45	7.45	7.45	7.45	0.05	7.45	0.027
	J	7.28	7.28	7.36	7.31	7.25	7.29	7.29	7.31	7.22	7.22	0.14	7.29	0.042
6	A	7.80	7.82	7.85	7.79	7.82	7.85	7.87	7.87	7.88	7.88	0.09	7.83	0.033
	J	7.71	7.80	7.73	7.78	7.68	7.70	7.70	7.67	7.74	7.74	0.13	7.73	0.050
7	A	9.45	9.38	9.44	9.40	9.35	9.33	9.33	9.43	9.43	9.43	0.12	9.39	0.044
	J	9.23	9.18	9.33	9.28	9.30	9.28	9.28	9.24	9.35	9.35	0.17	9.27	0.086
Measurements made on 1:5 "Soil-Water Suspension"														
3	A	7.96	7.95	7.93	7.92	7.93	7.92	7.92	7.94	7.93	7.93	0.04	7.94	0.014
	J	7.68	7.73	7.77	7.77	7.74	7.74	7.74	7.79	7.70	7.70	0.11	7.74	0.037
6	A	8.45	8.43	8.40	8.47	8.45	8.44	8.44	8.41	8.43	8.43	0.06	8.44	0.023
	J	8.26	8.35	8.37	8.33	8.34	8.34	8.34	8.23	8.24	8.24	0.14	8.31	0.048
7	A	10.25	10.23	10.28	10.23	10.27	10.30	10.30	10.28	10.24	10.24	0.07	10.26	0.026
	J	10.20	10.08	10.11	10.12	10.11	10.09	10.09	10.03	10.18	10.18	0.17	10.12	0.055

samples and justifies, therefore, a standardization of the method for determining the reaction of alkaline soils.

#### DIFFERENCES AMONG ANALYSTS AND IN SOIL-WATER RATIOS

As significant differences between analysts and interaction effects were not quite so apparent, it was thought desirable to submit the data in Table 1 to an analysis of variance according to the method of Fisher.<sup>1</sup> The results obtained are shown in Table 5.

*Variation with analysts.*—The variance in the means of analysts is highly significant. As a consequence, the data submitted by each analyst were also tested for significance against those submitted by the other analysts. The method used was one proposed by Brandt<sup>2</sup> for a "Unique Sample," and the results obtained are shown in Table 6. The results of Analyst I are not considered here.

It may be noted that the results submitted by Analyst D are generally significantly lower than those submitted by the other analysts and that the results submitted by Analysts C and G are in each of four cases significantly higher than those of the other analysts. The greatest mean difference, equal to 0.086 pH units, is between Analysts D and G. It is interesting that the differences in all the results should be within an average pH range of only 0.186 pH units, and yet be so consistent that a statistically highly significant variance was obtained. This would indicate that the procedures at the different laboratories for making the pH measurements were rigorously controlled; otherwise more variable results within this narrow pH range would have been obtained, with subsequent lack of significance.

The analyst's sample interactions were significant, indicating that the method used by the different analysts was independent of or not influenced by the soil used

*Variation with soil-water ratios.*—The data in Table 1 show clearly that a marked difference existed between the pH values obtained on the moist soils and those obtained on 1:5 soil-water suspensions. In every instance the value obtained on the diluted sample was higher than that taken at field moisture. The mean differences for the alkaline soils ranged from 0.400 pH units to 0.838 units, with an average mean difference of 0.586 pH units. As was emphasized last year, therefore, if pH determinations made at different laboratories on alkaline soils are to be properly comparable, the soil-water ratios at which the determinations are made must be standardized.

Reference to Table 5 also shows that the sample:soil-water ratio interaction was highly significant, thus emphasizing the fact that the increase in pH with dilution varies with the soil sample being tested. This is evi-

<sup>1</sup> R. A. Fisher, *Statistical Methods for Research Workers*, Chap. VII. Oliver and Boyd, London (1936).

<sup>2</sup> A. E. Brandt, *J. Am. Stat. Assoc.*, 28, 434-436 (1933).

dent from Table 1, in which the results for the different samples were recorded in the ascending order of their mean  $pH$  values and the numbers in parentheses, (1) to (9), assigned to them. The results obtained on the 1:5 soil-water suspensions were also lined up in an ascending mean  $pH$  order. It may be noted that the position of one sample in relation to another one was far different in this latter case than when the measurements were made at field moisture content. For example, Sample 2 now holds the 6th position and Sample 7 the 9th position and so on. It seems to the writers that this emphasizes the need for standardization of the soil-water ratio at which  $pH$  measurements are to be made if results are to

TABLE 5.—*Analysis of variance of pH values for different western soils as reported by various analysts (two soil-water ratios used)*

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between means of samples	8	
Between means of analysts	8	0.0673†
Between means of soil-water ratios	1	10.6656†
Interactions:		
Analyst: soil-water ratios	8	0.0074
Analyst: samples	64	0.0210
Samples: soil-water ratios	8	0.1212†
Error	58	0.0145
Total	155*	

\* Refers to a deduction of 6 degrees of freedom for estimated values.

† Refers to a highly significant difference.

be comparable, and also for a ratio selected with a view to the application of the results to soil fertility studies. Inasmuch as crops grow in soils principally at the lower moisture content and not in 1:5 soil-water suspension, the more representative  $pH$  would be the one taken at field moisture content.

Heretofore, the objection to  $pH$  determinations at low moisture content has been the difficulty of obtaining good contact between the electrode and the soil sample being tested, with resulting inaccuracy of measurement. However, with the advent of the improved glass electrode used in these cooperative studies, eight different analysts reported results that were no more variable than those obtained on a 1:5 soil-water suspension and whose significant range (mean plus and minus the standard deviation) was but 0.22  $pH$  units. Objections to making  $pH$  measurements on soils at low moisture content on a basis of inaccuracy of measurement, therefore, are no longer tenable. And if it is true that such  $pH$  values represent more nearly the average conditions present in the soil under natural con-

ditions, then serious consideration should be given to adopting the field moisture content as proper for the pH measurements in alkaline soils.

### SUMMARY AND CONCLUSIONS

Nine soils from widely separated sections of the West were collected and analyzed by 10 collaborators in several of the Western States. It was specified that the glass electrode be used for the pH determinations and that each instrument be standardized against a buffer or one of the soil samples. One series of determinations was made at a low moisture content (approximately the moisture equivalent) and another at a 1:5 soil-water ratio.

TABLE 6.—*Differences between the mean pH values of several western soils as reported by various analysts*

ANALYST	MEAN pH VALUE	MEAN pH DIFFERENCES FOR THE FOLLOWING ANALYSTS—								
		A	B	C	D	E	F	G	H	K
A	8.299	0	—	—	—	—	—	—	—	—
B	8.339	−0.040	0	—	—	—	—	—	—	—
C	8.419	−0.121†	−0.080*	0	—	—	—	—	—	—
D	8.238	0.061*	0.101*	0.181†	0	—	—	—	—	—
E	8.333	−0.034	0.006	0.086	−0.095*	0	—	—	—	—
F	8.382	−0.083	−0.043	0.037	−0.144†	−0.049	0	—	—	—
G	8.414	−0.115*	−0.075	0.005	−0.186†	−0.081	−0.032	0	—	—
H	8.317	−0.018	0.022	0.102*	−0.079	0.016	0.065	0.097*	0	—
K	8.367	−0.068	−0.028	0.052	−0.129*	−0.034	0.015	0.047	−0.050	0

\* Refers to a significant mean difference.

† Refers to a highly significant mean difference.

The data obtained from this year's collaborative work give confirmative support to the method proposed on the basis of last year's work; namely, that the pH determinations be made on semi-arid soils at a low moisture content.

The little or no difference in the pH value at the moisture equivalent and the water holding capacity shows that there is no need for great accuracy in adding water to the soil as long as the moisture content is held below the water holding capacity. This condition greatly simplifies the method.

A comparison of methods of wetting the soil favors the mixing of soil and water in a 50 ml. beaker.

The data submitted by the different analysts are comparatively uniform; pH values obtained on the soils at field moisture content are less variable than those obtained at a 1:5 soil-water ratio, the average standard deviations of the data being 0.11 and 0.14 pH units, respectively. This is slightly higher than the variation obtained by a single analyst on several aliquots of the same soil sample but falls within the 0.12 variation thought desirable.

The data were submitted to an analysis of variance to determine whether or not significant differences exist between the results submitted by the different analysts and to isolate any interaction effects that may be operative. It was found that significant differences do exist between the results obtained by the different analysts. Small but consistent differences in technic were apparently used at the different laboratories.

The variance between means of  $pH$  values at the different soil-water ratios is also highly significant. The average apparent increase in  $pH$  with dilution, 0.586  $pH$  units, emphasizes the need for standardization of the soil-water ratios at which the  $pH$  determinations are to be made if results from the different laboratories are to be comparable.

The sample:soil-water ratio interaction was also highly significant. It indicates that the increase in  $pH$  with dilution varies with the soil sample being tested.

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No report on the hydrogen-ion concentration of soils of humid regions was given by the associate referee.

## REPORT ON LIMING MATERIALS

### SOLUBILITIES OF CALCIC AND MAGNESIC LIMESTONES IN NEUTRAL NORMAL AMMONIUM ACETATE

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

In the determination of replaceable bases by extraction of soils with neutral salt solutions it is not feasible to distinguish between calcium and magnesium derived from replaceable form and from carbonate form, although replaceable  $Ca + Mg$  can be determined readily by effecting complete removal of both replaceable and carbonate contents with boiling ammonium chloride solution and application of correction for carbonate content.

Ammonium acetate extractions of soils, with and without corrections for carbonate, are being used in the determination of replaceable bases. Little attention, however, has been given to ultimate solubilities or to the relative rates of dissolution of calcic and magnesian limestone in the acetate extractant. Nevertheless, a knowledge of such relative dissolution rates may suggest experimental conditions whereby one of the several factors in a complex soil system will be minimized to such an extent as to facilitate interpretation of the analytical results.

The objective of this report is to present data on the dissolution rates of calcic and magnesian limestones in neutral normal ammonium acetate, to establish the ultimate solubilities of such limestones, and thereby to

simplify the determination of replaceable calcium and magnesium to the exclusion of carbonates.

### EXPERIMENTAL

The charges of limestone and 100 ml. of neutral normal ammonium acetate were placed in 250 ml. Erlenmeyer flasks, which were stoppered with rubber stoppers and shaken on a rotary shaking machine for the prescribed period. Upon completion of the shaking period, the suspension was filtered on a gravity filter, the first 10–15 ml. being discarded, and a 50 ml. aliquot of the clear solution was evaporated to dryness in a 400 ml. Pyrex beaker on a hot plate. The residue was then incinerated in an electric furnace at 550° C., for at least 10 minutes. The residue was cooled and dissolved by the addition of measured excess of 0.1 *N* hydrochloric acid, diluted with 5–10 ml. of distilled water, and digested on a hot plate until the bottom of the beaker was free of crusty material. The excess of acid was titrated with 0.1 *N* sodium hydroxide against methyl orange. The net acid titer times 2 was taken as a measure of the total bases dissolved. In some instances the calcium and magnesium contents of the titrated solutions were also determined.

### RATES OF DISSOLUTION OF CALCIC AND MAGNESIC LIMESTONES

For this set of experiments, 0.25 gram charges of either limestone or dolomite of varying fineness were shaken for different periods to observe any difference in the progress of the dissolution of the two types of limestone. The results given in Table 1 definitely establish the rapidity of the

TABLE 1.—*Progress of dissolution of limestone and dolomite of varying fineness in neutral normal ammonium acetate*

AGITATION PERIOD	0.1 <i>N</i> EQUIVALENCE OF DISSOLVED AND TITRATED BASES IN 100 ML. OF SOLUTION					
	LIMESTONE (a)			DOLOMITE (a)		
	—60 +80	—200 +325	—325	—60 +80	—200 +325	—325
<i>hours</i>						
1	10.8	12.9	13.4	.3	.9	3.0
3	12.3	13.4	13.5	.9	1.8	—
8	13.2	13.4	13.4	1.8	3.0	4.6
16	13.4	13.6	13.6	2.5	3.9	5.8
24	13.4	13.6	—	3.0	4.3	6.5
48	13.6	13.6	—	4.1	6.0	7.4
72	13.6	13.6	—	5.4	5.6	6.9
120	13.6	13.6	—	4.8	7.1	8.4
240	—	—	—	—	—	13.6*
360	—	—	—	—	—	13.7*
480	—	—	—	—	—	14.5*
600	—	—	—	—	—	15.2*

(a) Analytical charge of 0.25 gram, except those designated by asterisk.



dissolution of calcic limestone against that of magnesian limestone. The coarser the two types of limestone and the shorter the period of contact, the greater was the divergence in speed of dissolution. This is easily understandable since it can be observed that the calcic limestone of 60–80-mesh approaches a solubility maximum within the 8 hour agitation period, and that of the 200–325-mesh separate attains the same constant within the 3 hour period. This solubility of 0.068 gram of calcium carbonate equivalence in 100 ml. neutral ammonium acetate is for a crystalline calcic limestone at room temperature of approximately 25° C., and will vary considerably with change in the temperature of the extractant. On the other hand, dissolution of the dolomitic limestone begins at a very low rate, and continues indefinitely within the 600 hour limits of this experiment. When the extraction of calcic limestone is completed—8 and 3 hours, respectively, for the 60–80-mesh and 200–325-mesh separates—the corresponding separates of magnesian limestone are dissolved to the extent of 1 to 7.5 of the calcic limestone. At the 1 hour period this solubility ratio is 1 to 36 and 1 to 14 for the 60–80- and 200–325-mesh separates, respectively.

An attempt was made to establish a solubility maximum for the magnesian limestone by increasing the charge and decreasing the particle size. A 0.25 gram charge of the magnesian limestone of –325-mesh, attained after 120 hours, a solubility  $\frac{2}{3}$  that of the calcic limestone of like charge and fineness from only 1 hour of agitated suspension. When the charge was increased to 1 gram and the periods of contact were extended to 600 hours, the solubility values shown by the magnesian limestone exceeded those shown by the calcic limestone, but without indication of attaining any limiting value. From the results of these limited experimental conditions, it appears that the relative solubilities of calcic and magnesian limestones in ammonium acetate may be made wide or narrow, dependent upon experimental variables, such as degree of fineness, size of charge, and period of contact.

#### EFFECT OF TEMPERATURE ON THE DISSOLUTION OF CALCIC AND MAGNESIAN LIMESTONE IN AMMONIUM ACETATE

For this experiment 0.25 gram charges of limestone per 100 ml. of ammonium acetate were taken. To facilitate utilization of a room provided with temperature control, the Ross-Kershaw shaker was used instead of the large rotary shaker generally employed. The contact period of this experiment was 16 hours. From the results shown in Table 2, it appears that a decrease in temperature has a substantially greater effect on speed of the dissolution of the magnesian limestone, although the effect upon the solubility of the calcic limestone was appreciable. It is to be expected that changes of several degrees in the room temperature may result in appreciable deviation in results of replicated experiments carried out at dif-

TABLE 2.—*Effect of temperature on dissolution of calcic and magnesian limestone in neutral normal ammonium acetate*

LIMESTONE		0.1 N EQUIVALENCE OF DISSOLVED AND TITRATED BASES IN 100 ML. OF SOLUTION	
TYPE	FINESS	25° C.	11° C.
Calcic	—200 + 325	14.0	10.9
Magnesian	—60 + 80	3.0	.8
Magnesian	—200 + 325	4.0	1.6
Magnesian	—325	6.2	3.3

ferent seasons. More exact information on the effect of temperature upon solubilities will have to await facilities for shaking for longer periods at constant temperatures.

#### DISSOLUTION OF LIMESTONE AND DOLOMITE FROM LIMITED CHARGES

The 0.25 gram and 1 gram charges used in the preceding experiments were in excess of the saturation capacity of 100 ml. of ammonium acetate. In the following experiments charges of either calcic or magnesian limestone were limited to 0.05 gram, which, under conditions of maximal dissolution of the calcic material, would be equivalent to 9.9 ml. titration of 0.1 N. The suspensions were agitated for 16 hours, which is a convenient overnight period commonly used for soil extractions. The limestones used were prepared by passage through 40-, 60-, and 100-mesh sieves, all of the fines being included. The question to be answered by this experiment was: With charges fixed at 0.05 gram and the agitation period to 16 hours, (a) what sieve fineness would be requisite to assure complete dissolution of the calcic limestone in 100 ml. of ammonium acetate, and (b) what would be the solubility of magnesian limestones under like conditions?

The results given in Table 3 show a 0.2 ml. shortage of complete disso-

TABLE 3.—*Dissolution from 0.05 gram charges of calcic and magnesian limestone of varying fineness in neutral normal ammonium acetate*

MATERIAL AND SOURCE	0.1 N EQUIVALENCE OF DISSOLVED AND TITRATED BASES IN 100 ML. OF SOLUTION		
	—40	—60	—100
Calcic limestone—Appalachian	9.3	9.7	9.7
Magnesian limestone—Am. Limestone Co.	1.6	1.6	2.0
Magnesian limestone—James River Co.	1.5	1.6	1.8
Magnesian limestone—Kelley Island Co.	1.8	2.0	2.4
Magnesian limestone—Standard Lime Co.	1.5	2.0	2.2
Magnesian limestone—Warner Lime Co.	1.6	1.8	2.0
Magnesian limestone—Lodd Lime Co.	1.4	1.6	2.2
Magnesian limestone—Averages	1.6	1.8	2.1

lution of the charges of 60- and 100-mesh calcic limestone. If this apparent deficiency is considered to be within experimental error the results indicate that a 16-hour agitation with 100 ml. of ammonium acetate will effect removal of a 0.5 per cent content of calcic limestone from a 10 gram charge of soil prepared by passing through a 60-mesh sieve. Under similar conditions as to charge and period of agitation, the average solubility of 6 dolomites gives a titration value of 1.8 ml. It follows that the maximal solubility effect in the ammonium acetate extraction of a 10 gram charge of soil carrying as high as 0.5 per cent of dolomite would amount to about 1 milliequivalent of magnesium per 100 grams of soil. Greater solubility effects will, of course, be obtained from larger proportions of dolomite in the soil.

#### EFFECT OF ADDED SOIL ON THE DISSOLUTION OF CALCIC AND MAGNESIC LIMESTONE

In this experiment 0.05 gram charges in 100 ml. of ammonium acetate were shaken 16 hours as before, except that in addition to the limestone there was also added 10 gram charges of a slightly acidic Etowah silt loam. The extraction technic was modified further by filtration on a Büchner filter and washing with ammonium acetate to a volume of 250 ml. This treatment was more drastic than the mere extraction of the limestones with 100 ml. of ammonium acetate, but was made to conform to the regular procedure for soils. The results are given in Table 4. It will be

TABLE 4.—*Effect of soil on the dissolution of calcic and magnesian limestone and limited charge*

MESH	0.1 N EQUIVALENCE OF DISSOLVED BASE FROM—					
	CALCIC LIMESTONE			MAGNESIC LIMESTONE		
	ALONE	WITH SOIL	CORRECTED	ALONE	WITH SOIL	CORRECTED
—40	9.3	15.9	9.5	1.8	8.1	1.7
—60	9.7	16.2	9.8	2.0	8.2	1.8
—100	9.7	16.1	9.7	2.4	8.8	2.4

seen that under these particular conditions the inclusion of the soil had little effect on the dissolution of the charges of the limestones. The previous conclusions as to solubility of calcic limestone and the solution effect of magnesian limestone in the soil system has been corroborated by these additional results. It may be anticipated that both the acidity of the soil and the attrition exerted by the soil mass during agitation are of some effect in increasing the speed with which both kinds of limestone undergo dissolution, especially for occurrences of the calcic type in proportions greater than those imposed in the present experiment.

### EFFECT OF THE PRESENCE OF $\text{CaCO}_3$ UPON THE DISSOLUTION OF MAGNESIC LIMESTONE

In the foregoing experiments the solvent effects of ammonium acetate upon calcic and magnesian limestones were investigated with only one or the other in the systems studied. In the following experiment an attempt was made to evaluate the dissolving capacity of ammonium acetate upon magnesian limestone in the presence of varying proportions of calcic limestone. The materials were of 100 mesh and the agitation period was 16 hours. The quantities and the proportions of the limestones used per 100 ml. of ammonium acetate, together with the results, are given in Table 5.

TABLE 5.—*Effect of the presence of  $\text{CaCO}_3$  on the solubility of magnesian limestone*

LIMESTONE ADDED PER 100 ML. OF AMMONIUM ACETATE		0.1 N EQUIVALENCE OF DISSOLVED AND TITRATED BASES IN 100 ML. OF SOLUTION		
TOTAL	CALCIC: MAGNESIC RATIO	TOTAL	Ca	Mg
gram				
0.05	4:1	7.6	7.7	.10
.05	3:2	5.8	5.8	.10
.05	2:3	4.5	4.4	.20
.05	1:4	3.5	2.7	.80
.10	1:1	9.9	9.9	.10
.20	1:1	14.0	14.0	.20

It appears that if the ratio of calcium carbonate to dolomitic limestone is 1:1 or wider in total charges from 0.05 to 0.2 gram, no appreciable dissolution of the dolomite will take place. The most useful application of these findings to base exchange studies is the possibility of extracting the replaceable magnesium of a soil by ammonium acetate without interference from dolomitic limestone by the simple expedient of adding 0.05 gram of calcium carbonate per 100 ml. of ammonium acetate.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That further work be done on the extraction of exchangeable calcium and magnesium in the presence of carbonates.
- (2) That studies on the direct determination of exchangeable hydrogen in soils be continued.

\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 48 (1941).

## REPORT ON LESS COMMON METALS IN SOILS

### COMPARISON OF PERCHLORIC ACID DISTILLATION AND SODIUM CARBONATE FUSION FOR DETERMINATION OF TOTAL BORON IN SOIL\*

By J. S. McHARGUE, *Associate Referee* and W. S. HODGKISS (Department of Chemistry, Kentucky Agricultural Experiment Station, Lexington, Ky.)

During the past year methods for the determination of boron in soils and siliceous materials were studied. The procedure of MacIntire and Hammond, *This Journal*, 22, 231 (1939), for the separation of fluorine from soils suggested the possibility of a similar procedure for the volatilization of boron from its compounds in the soil. The procedure that has been followed in this laboratory, together with tables of experimental data, are presented in this report.

#### TOTAL BORON IN SOILS

##### REAGENTS

- (a) *Hydrochloric acid*.—0.36 *N* (approximate). 10 ml. of 96%  $H_2SO_4$  per liter.
- (b) *Quinalizarin-sulfuric acid indicator solution*.—98.5% by weight  $H_2SO_4$  containing 0.005 gram of quinalizarin per liter.
- (c) *Standard boron solution*.—1.1432 grams of  $H_3BO_3$  per liter of 0.18 *N*  $H_2SO_4$ . Dilute so that a series of standards contains 0.000–0.005 microgram of B per ml. in 0.18 *N*  $H_2SO_4$ .

##### PROCEDURE

Mix 0.5 gram of finely ground sample of soil with 1.5 grams of precipitated  $CaO_2$  in either a nickel or platinum crucible. Char thoroughly and incinerate at approximately 900° C. for 30 minutes. Cool, and place in the distillation flask of the apparatus suggested by Willard and Winter, *This Journal*, 16, 105 (1933). Add 5 ml. of a solution that contains 0.05 mg. of fluorine and neutralize to phenolphthalein with 60%  $HClO_4$ , adding 10 ml. in excess. Distil at  $135^\circ \pm 5^\circ$  C., collecting a minimum of 225 ml. of distillate kept alkaline to phenolphthalein by  $K_2CO_3$ . Evaporate the distillate to dryness in platinum and heat at 450° C. for 30 minutes.

Add 25 ml. of the 0.36 *N*  $H_2SO_4$  and mix thoroughly. Run in aliquot of 1 ml. of the clear solution into a comparison tube and add 9 ml. of the acid-indicator solution. Mix thoroughly, stopper, and allow to stand for 30 minutes before comparing with standard boron solutions.

#### EXPERIMENTAL

Since fluorine in large excess will interfere with the quinalizarin determination of boron, a series of experiments was made to determine the effect of the quantities of fluorine that might be expected to be found in the final aliquot of the concentrated distillate. Table 1 shows that an error of 2.0 micrograms of boron might be expected when 0.05 mg. of fluorine was present in the aliquot. This would be equivalent to an error of 0.25 per cent in the original sample.

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\* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

TABLE 1.—*Error in colorimetric determination of boron with fluorine in the solution*

SAMPLE NUMBER	F PRESENT PER ALIQUOT	APPARENT BORON PER ALIQUOT	PLUS ERROR IN BORON INDUCED BY PRESENCE OF F
	microgram	microgram	microgram
1	0.005	0.00	0.0
2	0.010	0.00	0.0
3	0.015	0.05	0.2
4	0.020	0.10	0.5
5	0.025	0.30	1.5
6	0.050	0.40	2.0

Preliminary experiments showed that rather erratic results were obtained from a direct distillation of the incinerated soil. However, addition of excess fluorine as NaF aided in the recovery of total boron present in the soil as measured by a fusion of another portion of the same soil.

Similar experiments showed that a large volume of distillate was required. Table 2 shows the variation in boron content of distillates from

TABLE 2.—*Recovery of boron on distillation*

SAMPLE NUMBER	VOLUME OF DISTILLATE	B—AIR DRY SOIL
	ml.	p.p.m.
1	50	1.8
2	75	0.3
3	100	3.0
4	125	13.0
5	150	35.0
6	175	22.6
7	200	44.0
8	225	55.5
9	250	56.0

the same soil. A sodium carbonate fusion of this soil, extraction with alcohol, and subsequent colorimetric determination gave a value of 55

TABLE 3.—*Total boron in soils by distillation and fusion procedure*

NUMBER	SOIL DEPTH	B OF AIR DRY SOIL (P.P.M.)		
		WATER-SOLUBLE BORON	TOTAL BORON FUSION	TOTAL BORON DISTILLATE
289	Surface	0.75	27.0	26.5
933A	0- 8"	2.17	55.0	56.0
933B	8-17"	0.93	62.0	64.5
933C	17-34"	0.89	51.5	54.0
933D	34-66"	1.05	44.5	40.5
933E	66-72"	1.23	68.0	67.0

p.p.m. of total boron in the air-dry soil. A minimum of 225 ml. of distillate is necessary for recovery of the total boron (Table 2).

A series of samples was then compared as to water-soluble boron and total boron according to the Berger-Truog procedure<sup>1</sup> and total boron by the perchloric acid distillation procedure. These data show good correlation between the fusion and distillation procedures. Some variations occur but they are not consistent in any one direction.

It is recommended\* that the perchloric acid distillation procedure be studied further, especially for the purpose of ascertaining boron and fluorine in the same sample of soil.

## REPORT ON FERTILIZERS

By G. S. FRAPS (Agricultural Experiment Station,  
College Station, Texas), *Referee*

Vitamin B<sub>1</sub> is a new addition to the list of fertilizer ingredients. It is used in fertilizers intended for flower and home gardens, but not those used on farm lands. Highly exaggerated claims regarding the value of this vitamin have been made. It seems to be needed in the growth of roots, but it is formed in the leaves. With most plants sufficient quantities are supplied to the roots from the leaves. The roots of some slow growing plants may not receive sufficient amounts of vitamin B<sub>1</sub> for rapid growth, and supplementary additions may increase the rate of growth of both roots and leaves. Vitamin B<sub>1</sub> may also be beneficial for the development of roots at the time of transplanting and may prevent the loss of some plants. Methods for the determination of vitamin B<sub>1</sub> have been devised for foods, and these methods may be applied to fertilizers when the need arises.

This Association has two methods for the determination of water in fertilizers, one by loss of weight in drying and the other by direct measurement of the water distilled off. It is obvious that measurement by loss in weight by drying is incorrect if appreciable quantities of other volatile substances are also lost. It has been reported that losses of as much as 2 per cent ammonia may occur when some samples of wet fish scrap are dried. The Associate Referee on Phosphoric Acid, W. H. Ross, is studying both methods, and the matter has also been studied by the Associate Referee on Nitrogen, A. L. Prince.

A special rubber stopper for use in Kjeldahl distillations is now on the market. It lasts much longer than do ordinary stoppers. The specifications are as follows: Diameter of small end, 32 mm.; diameter of large end, 37 mm.; length, 36 mm., with one hole 6 mm. in diameter. They may be secured from the Central Scientific Company of Chicago.

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 540 (1939).

\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 48 (1941).

Recommendations regarding changes in methods of analysis, which are approved by the Referee, will be made by the associate referees.

No report on phosphoric acid was given by the Associate Referee, William H. Ross, because he was assigned another topic, that of Sampling of Fertilizers. A paper describing the results of this study follows.

## PREPARATION OF FERTILIZER SAMPLES FOR ANALYSIS

By WILLIAM H. ROSS, L. F. RADER, JR., and JOHN O. HARDESTY  
(Fertilizer Research Division, Bureau of  
Plant Industry, Washington, D.C.)

The present official method for sampling fertilizers in the field was adopted at the 36th meeting of this Association in 1920 (2) on the recommendation of a committee appointed by this Association to study the sampling of fertilizers in cooperation with a similar committee from the American Chemical Society. The method for preparing the field samples for analysis in the laboratory was adopted 31 years earlier at the sixth meeting of the Association in 1889 (5). The wording of the method as it first appeared in the 1919 edition of *Methods of Analysis, A.O.A.C.* was as follows:

Reduce the gross sample by quartering to an amount sufficient for analytical purposes. Transfer to a sieve having circular openings 1 mm. in diameter, sift, breaking the lumps with a soft rubber pestle. Grind in a mortar the part remaining on the sieve until the particles pass through. Mix thoroughly and preserve in tightly stoppered bottles. Grind and sift as rapidly as possible to avoid loss or gain of moisture during the operation.

Long experience has shown that this method of preparing fertilizer samples in the laboratory gives satisfactory results in the analysis of materials as well as of mixtures that exhibit little or no tendency to segregate. Within recent years, however, the property of fertilizers to undergo segregation has shown an upward trend for the reasons explained in an accompanying paper, entitled "Sampling of Fertilizers" (see p. 499). This property of fertilizers has increased the difficulty of securing uniform samples for analysis, and the claim has frequently been made (1, 3, 5) that finer grinding than that directed in the official method is necessary if concordant results are to be obtained in the analysis of certain types of mixtures and particularly of high analysis mixtures. At the last meeting of this Association, a recommendation was accordingly adopted that a collaborative study be made of the fineness to which fertilizers should be ground in the preparation of samples for analysis (4). The present report gives the results obtained in the study that was undertaken in compliance with this recommendation.



## STANDARD SAMPLES

Eight mixed fertilizer samples were prepared for this collaborative study with a view to showing (a) to what extent segregation may affect the analysis of samples that have been ground to pass a 1 mm. sieve; and (b) the degree of grinding necessary to offset this effect if found to be significant. Two of the samples were 4-8-4 mixtures prepared from commercial materials, and six were 8-16-16 mixtures prepared from C.P. materials. As segregation is not likely to take place in caked mixtures, an attempt was made to prepare samples that would undergo little or no caking on standing. The formula of the 4-8-4 mixtures and the range of particle size of the materials used in their preparation are given in Table 1,

TABLE 1.—*Formulas of 4-8-4 mixtures (Samples 1 and 2)*

	PARTICLE SIZE IN SAMPLE		AMOUNT TAKEN FOR EACH SAMPLE
	1	2	
	<i>mesh</i>	<i>mesh</i>	<i>grams</i>
Ammoniated Superphosphate, N = 2.97%; $P_2O_5$ = 20.70%	100-150	100-150	24.50
Ammonium Sulfate, N = 20.69%	40-60	40-60	6.87
Milorganite, N = 6.19%; $P_2O_5$ = 2.59%	1 mm.-30	<35	6.00
Potassium Sulfate, $K_2O$ = 45.50%	1 mm.-30	<35	5.54
Dolomite	40-60	40-60	9.30
Filler (Quartz Sand)	1 mm.-30	<35	7.79
Total			60.00

and the corresponding data for the 8-16-16 mixtures are given in Table 2.

The composition of the materials used in the preparation of the 4-8-4 mixtures was determined by chemical analysis. The analytical values given in Table 1 for the composition of these commercial materials represent

TABLE 2.—*Formulas of 8-16-16 mixtures (Samples 3-8)*

	PARTICLE SIZE IN SAMPLE						AMOUNT TAKEN FOR EACH SAMPLE
	3	4	5	6	7	8	
	<i>mesh</i>	<i>mesh</i>	<i>mesh</i>	<i>mesh</i>	<i>mesh</i>	<i>mesh</i>	<i>grams</i>
Monoammonium Phosphate, N = 12.17%; $P_2O_5$ = 61.70%	1 mm.-30	40-60	100-150	<35	40-60	100-150	15.56
Ammonium Sulfate, N = 21.20%	40-60	1 mm.-30	40-60	40-60	<35	40-60	13.71
Potassium Sulfate, $K_2O$ = 54.05%	100-150	100-150	1 mm.-30	100-150	100-150	<35	17.76
Filler. (Quartz Sand)	100-150	100-150	100-150	100-150	100-150	100-150	12.97
Total							60.00

the mean of the analytical results obtained by two of the authors. On the basis of these results, the 4-8-4 mixtures (Samples 1 and 2) were formulated to contain 4.20 per cent of nitrogen, 8.71 per cent of total phosphoric acid ( $P_2O_5$ ), and 4.20 per cent of potash. Sample 2 differed from Sample 1 only in that the milorganite, potassium sulfate, and filler, which had a particle size of 1 mm.-30 mesh when used in the preparation of Sample 1, were ground to pass a 35-mesh Tyler Standard sieve before being used in the preparation of Sample 2. Sample 1 was thus prepared to pass a 1 mm. perforated sieve as directed in the official method while Sample 2 passed a 35-mesh sieve.

The 8-16-16 mixtures were prepared from C. P. materials, and therefore their true composition could be determined independently of any method of analysis. Results obtained in the chemical analysis of these materials checked closely with the theoretical values. The mixed fertilizer samples (Samples 3-8) were formulated on the basis of these values to contain 8.00 per cent of nitrogen, 16.00 per cent of total phosphoric ( $P_2O_5$ ), and 16.00 per cent of potash.

All samples sent to the collaborators were prepared separately in order to avoid any difference in composition due to segregation. To reduce chance of error in the preparation of individual samples, each material of a given mesh size was weighed out by one of the authors under the observation of another author into all the bottles to be sent to the collaborators. The contents of each bottle were then subjected to thorough mixing on a glazed paper and returned to the bottle.

#### DIRECTIONS FOR ANALYSIS

- A. Determine nitrogen in Samples 1 and 2 as directed for organic and ammoniacal nitrogen in the 1935 edition of *Methods of Analysis, A.O.A.C.*, pages 23-25, Sections 19-25. Determine nitrogen in Samples 3-8 as directed for ammoniacal nitrogen in the 1935 edition of *Methods of Analysis, A.O.A.C.*, page 26, Section 30.
- B. Determine total  $P_2O_5$  in each mixture as directed in the 1935 edition of *Methods of Analysis, A.O.A.C.*, pages 19-21, Sections 7-12.
- C. Determine potash in each sample as directed in the 1935 edition of *Methods of Analysis, A.O.A.C.*, pages 29-30, Sections 42-44(a).

#### Notes

1. It is suggested that each sample be mixed on a paper or other suitable material before the required quantity is withdrawn for analysis, but the samples should not be ground or otherwise treated in any way.
2. A separate weighing should be made for each determination.
3. If it is not convenient to analyze all the samples, it is suggested that Samples 3 and 6, and/or Samples 4 and 7, and/or Samples 5 and 8 be discarded.
4. In submitting a report on the work, it is important that all results be given rather than the mean of replicated results.

The following analysts collaborated in this work.

## COLLABORATORS

1. Austin, W. R., Armour Fertilizer Works, Nashville, Tenn.
2. Batton, H. C., Swift and Co., Fertilizer Works, Baltimore, Md.
3. Butt, C. A., Intern. Agr. Corp., East Point, Ga.
4. Caldwell, Paul, and Hoffman, A. E., Darling and Co., East St. Louis, Ill.
5. Caldwell, R. D., Armour Fertilizer Works, Atlanta, Ga.
6. Carpenter, F. B., Allen, A. H., Morris, R. M., Lazarus, Sam, and Powell, R. O., Virginia-Carolina Chemical Corp., Richmond, Va.
7. Charlton, R. C., American Agricultural Chemical Co., Carteret, N. J.
8. Cox, Alvin J. and Marshall, W. G., Dept. of Agriculture, Sacramento, Calif.
9. Howes, C. C., Davidson Chemical Corp., Baltimore, Md.
10. Ingham, R. E., F. S. Royster Guano Co., Macon, Ga.
11. Jones, W. Catesby, Dept. of Agriculture and Immigration, Richmond, Va.
12. Magruder, E. W., Lineweaver, A. N., Ryder, W. A., and Earnest, George, F. S. Royster Guano Co., Norfolk, Va.
13. Marshall, C. V., Carson, R. B., and Potvin, A., Dept. of Agriculture, Ottawa, Canada.
14. Neutzel, Carl, F. S. Royster Guano Co., Baltimore, Md.
15. Rader, Jr., L. F., Bureau of Plant Industry, Washington, D. C.
16. Shuey, P. McG., Shuey and Company, Savannah, Ga.

## RESULTS OF ANALYSIS

Table 3 gives the maximum, minimum, and mean, and the variation from the theoretical values of the results reported by the collaborators for total nitrogen, total phosphoric acid ( $P_2O_5$ ), and potash in each of the standard samples. Table 4 shows the theoretical values and the averages of all the results reported by the collaborators for N,  $P_2O_5$ , and  $K_2O$  in each sample. The standard deviations from the theoretical values of the results reported by all the collaborators for N,  $P_2O_5$ , and  $K_2O$  in each sample are given in Table 5.

## DISCUSSION OF RESULTS

Table 3 shows surprisingly wide variations between the replicated results for the samples that contained 1 mm.-30-mesh material (Samples 1, 3, 4, and 5). Although some of the duplicated results agree closely, there is often a wide difference between the mean of these results and the theoretical value. The only reasonable explanation for the wide variations in so many of the replicated determinations is that a marked segregation occurred in the mixtures in the process of weighing out the samples for analysis. The mixtures were purposely so prepared as to be subject to marked segregation, and being very dry they no doubt exhibited a greater tendency in this respect than would be true of most commercial mixtures. It was thought to be advisable, however, to prepare mixtures of this kind in order to leave no doubt as to the possible effect of segregation on the analysis of fertilizer mixtures. Mixtures that would not undergo any segregation could have been prepared but it was considered that nothing would be gained in analyzing samples of this kind. It may also be stated

that mixtures that would have shown a still greater tendency to segregate than those sent to the collaborators could have been prepared.

It is well known among analysts that when a spatula is used to withdraw samples, there is a tendency for a greater proportion of the large than of the small particles to roll off the spatula. If the larger particles consist mainly of one material and the small particles of another, then it might be expected that relatively low results would be found for constituents occurring in the coarse particles and high results for the constituents in the fine particles. If this statement is assumed to be true, then the results reported for  $P_2O_5$  in Samples 1 and 5 should be high relative to the  $K_2O$ ; the  $K_2O$  in Sample 3 should be high relative to the  $P_2O_5$ ; and the  $K_2O$  in Sample 4 should be high relative to both the  $P_2O_5$  and the N. An examination of the data is Table 4, which gives the averages of all the results reported by the collaborators for N,  $P_2O_5$ , and  $K_2O$  in each sample, shows that these relationships hold true in every case.

The manner in which the replicated results reported by the collaborators varied from the theoretical value for N,  $P_2O_5$ , and  $K_2O$  in each sample is shown by the data in Table 5. The standard deviations from theory for the finer-mesh mixtures (Samples 2, 6, 7 and 8) are less than for the coarser-mesh mixtures (Samples 1, 3, 4 and 5) with one exception. This exception occurs in the value found for  $P_2O_5$  in Sample 7, which is greater than that in the corresponding coarser-mesh mixture, Sample 4. The phosphate particles in Sample 7 were larger than those of any of the other components of the mixture, while in Sample 4 they were not so large as the ammonium sulfate particles. It might be expected, therefore, as was actually found, that a wider range of results would be obtained for  $P_2O_5$  in Sample 7 than in Sample 4 even though the average particle size of the latter sample was less than that of the former.

All samples contained two nitrogen carriers of different particle size, but only one carrier of  $P_2O_5$  and  $K_2O$ . It might be expected, therefore, that the nitrogen values would show better agreement than those for either  $P_2O_5$  or  $K_2O$ , and the data in Table 5 show this to be true in every case with the exception of Sample 4. Both of the nitrogen carriers in this sample were in the form of relatively coarse particles. Variable results with an average low value were, therefore, to be expected for nitrogen in this sample. This is in agreement with the mean of the results reported by the collaborators. Table 5 further shows that the most uniform results were obtained in the analysis of the 4-8-4 mixture that had been ground to pass a 35-mesh sieve (Sample 2). The results indicate that grinding to 35 mesh is sufficiently fine for the analysis of low-analysis mixtures, but that further grinding may sometimes be advisable in the case of high-analysis mixtures.

All the collaborative results show a mean total plant food content for the 8-16-16 mixtures of 40.16 per cent, which agrees very closely with the

theoretical value of 40.00 per cent. This close agreement affords further convincing evidence that the many wide variations in the results reported by the collaborators for N,  $P_2O_5$ , and  $K_2O$  in the samples were due to segregation and not to errors of analysis.

A preference for wire mesh sieves was indicated by 14 out of the 16 collaborators, and only two expressed a preference for sieves with circular openings. Five of the collaborators weighed out the samples for analysis directly from the bottle and 11 after mixing and spreading on a level surface. The results reported showed that neither procedure had any superiority over the other.

Most fertilizers are difficult to grind, and this is particularly true of mixtures that are moist or sticky. Mixtures of this kind, however, do not tend to segregate to the same extent as do those that are dry and have components that vary in their particle size. Many different devices are now being used for grinding fertilizers, such as the mortar and pestle, the Wiley mill, and various grinding and rolling mills. None of these is claimed to be entirely satisfactory for grinding all types of fertilizers. In many laboratories the sample is passed through a grinding mill, and the oversized particles remaining after one passage through the mill are ground with mortar and pestle.

The fineness to which fertilizers are ground for analysis varies greatly in different laboratories. In some laboratories the sample is ground to pass through a 1-mm. sieve as directed in the official method, while in others some materials at least are ground to 30-, 40-, 60-, or even 100-mesh. It is apparent from the results submitted in this collaborative study that dry fertilizers that vary in particle size should be ground for analysis to at least 35-mesh, but until some more efficient device is available for grinding moist fertilizer mixtures it would seem inadvisable to require that such mixtures be ground to a finer state of subdivision than that now prescribed by the official method.

#### RECOMMENDATION

It is recommended that fertilizer materials and moist fertilizer mixtures be ground for analysis to pass a 1-mm. sieve with circular openings, or a 20-mesh Tyler Standard sieve, and that dry mixtures that show a tendency to segregate may be ground to pass a 35-mesh Tyler Standard sieve.

#### SUMMARY

Fertilizer mixtures that pass a 1-mm. sieve may undergo sufficient segregation to affect seriously the results of analysis if the mixture is dry and its components differ in particle size. Good results are obtained in the analysis of the ordinary type mixtures that have been ground to pass a 35-mesh sieve. Most fertilizers are difficult to grind and this is particularly true of mixtures that are moist or sticky. Mixtures of this kind, how-

TABLE 3.—*Analysis of standard mixed fertilizer samples (per cent)*

COLLABORATOR	TOTAL NITROGEN				TOTAL PHOSPHORIC ACID (P <sub>2</sub> O <sub>5</sub> )				POTASH			
	HIGH	LOW	MEAN	VARIATION OF MEAN FROM THEORY	HIGH	LOW	MEAN	VARIATION OF MEAN FROM THEORY	HIGH	LOW	MEAN	VARIATION OF MEAN FROM THEORY
Sample 1 (4.20-8.71-4.20)												
1	4.40	4.16	4.27	+0.07	9.15	9.03	9.09	+0.38	4.38	4.19	4.28	+0.08
2	4.48	4.20	4.35	+0.15	9.83	9.32	9.50	+0.79	4.58	4.02	4.37	+0.17
3	4.55	4.14	4.32	+0.12	9.10	7.76	8.29	-0.42	3.89	2.59	3.41	-0.79
4	4.32	4.15	4.24	+0.04	10.10	9.65	9.85	+1.14	4.43	2.85	3.59	-0.61
5	4.27	4.08	4.19	-0.01	9.63	9.45	9.54	+0.83	4.23	3.97	4.11	-0.09
6	4.34	4.19	4.23	+0.03	10.75	8.93	9.51	+0.80	3.04	2.72	2.87	-1.33
7	4.45	3.95	4.28	+0.08	9.93	7.75	9.43	+0.72	4.69	3.49	4.16	-0.04
8	4.25	4.23	4.24	+0.04	9.78	9.01	9.38	+0.67	3.37	3.22	3.30	-0.90
9	4.40	4.30	4.35	+0.15	9.45	9.25	9.35	+0.64	4.50	4.03	4.27	+0.07
10	4.18	4.08	4.13	-0.07	9.45	8.85	9.08	+0.37	4.48	4.08	4.19	-0.01
11	4.50	4.15	4.26	+0.06	9.45	8.80	9.15	+0.44	4.80	4.11	4.41	+0.21
12	4.55	4.18	4.36	+0.16	10.05	8.92	9.42	+0.71	5.04	3.60	4.32	+0.12
13	4.48	4.30	4.41	+0.21	8.80	8.20	8.50	-0.21	4.02	3.11	3.57	-0.63
14	4.48	4.20	4.38	+0.18	10.30	9.30	9.80	+0.09	5.68	3.84	4.76	+0.56
15	4.54	4.26	4.36	+0.16	8.97	8.11	8.64	-0.07	4.99	3.01	4.11	-0.09
16	4.38	4.23	4.31	+0.11	9.42	9.28	9.35	+0.64	4.99	3.31	4.01	-0.19
Sample 2 (4.20-8.71-4.20)												
1	4.32	4.25	4.28	+0.08	9.10	9.00	9.05	+0.34	4.53	4.44	4.49	+0.29
2	4.37	4.17	4.29	+0.09	8.87	8.75	8.81	+0.10	4.48	4.40	4.44	+0.24
3	4.24	4.07	4.15	-0.05	8.97	8.75	8.89	+0.18	4.62	4.50	4.57	+0.37
4	4.23	4.18	4.20	0.00	9.20	9.00	9.10	+0.39	4.32	4.25	4.28	+0.08
5	4.29	4.16	4.22	+0.02	8.95	8.90	8.93	+0.22	4.64	4.43	4.50	+0.30
6	4.33	4.12	4.20	0.00	9.13	8.63	8.95	+0.24	4.50	4.42	4.46	+0.26
7	4.37	4.19	4.28	+0.08	9.00	8.90	8.93	+0.22	4.31	4.21	4.26	+0.06
8	4.40	4.34	4.37	+0.17	9.00	8.97	8.99	+0.28	4.39	4.18	4.29	+0.09
9	4.25	4.10	4.18	-0.02	9.03	9.00	9.02	+0.31	4.53	4.38	4.46	+0.26
10	4.27	4.20	4.23	+0.03	9.35	9.30	9.32	+0.61	4.56	4.52	4.54	+0.34
11	4.38	4.15	4.32	+0.12	9.05	8.90	9.00	+0.29	4.44	4.19	4.29	+0.09
12	4.35	4.28	4.32	+0.12	9.38	9.15	9.27	+0.56	4.56	4.48	4.54	+0.34
13	4.66	4.19	4.39	+0.19	8.60	8.48	8.55	-0.16	4.46	4.40	4.43	+0.23
14	4.63	4.40	4.52	+0.32	9.05	9.05	9.05	+0.34	4.60	4.48	4.54	+0.34
15	4.40	4.20	4.28	+0.08	8.94	8.73	8.84	+0.13	4.53	4.12	4.32	+0.12
16	4.48	4.06	4.23	+0.03	9.30	9.05	9.15	+0.44	4.53	4.48	4.51	+0.31

TABLE 3.—Continued

COLLAB-ORATOR	TOTAL NITROGEN				TOTAL PHOSPHORIC ACID (P <sub>2</sub> O <sub>5</sub> )				POTASH			
	HIGH	LOW	MEAN	VARIA- TION OF MEAN FROM THEORY	HIGH	LOW	MEAN	VARIA- TION OF MEAN FROM THEORY	HIGH	LOW	MEAN	VARIA- TION OF MEAN FROM THEORY
Sample 3 (8.00-16.00-16.00)												
1	8.15	7.86	7.99	-0.01	16.50	15.20	15.68	-0.32	16.44	15.74	16.26	+0.26
2	7.63	7.11	7.38	-0.62	17.87	15.15	16.73	+0.73	16.91	15.74	16.14	+0.14
3	8.34	7.12	7.82	-0.18	21.14	11.71	15.77	-0.23	17.85	15.80	16.64	+0.64
4	7.78	7.60	7.69	-0.31	16.40	15.60	15.97	-0.03	15.98	15.44	15.68	-0.32
5	7.42	7.17	7.29	-0.71	14.05	13.38	13.69	-2.31	17.34	16.73	17.02	+1.02
6	8.46	6.72	6.68	-1.32	16.68	15.75	16.21	+0.21	17.94	17.08	17.60	+1.60
7	8.72	7.49	8.06	+0.06	21.30	13.40	17.35	+1.35	17.13	14.12	15.38	-0.62
8	8.01	7.91	7.96	-0.04	18.46	16.25	17.26	+1.26	18.58	15.06	16.82	+0.82
9	8.00	6.95	7.48	-0.52	14.78	14.35	14.62	-1.38	16.32	15.80	16.06	+0.06
10	8.30	7.80	8.05	+0.05	16.90	16.60	16.80	-0.80	16.76	16.08	16.40	+0.40
11	8.50	8.00	8.20	+0.20	14.90	13.25	14.26	-1.74	17.39	15.95	16.69	+0.69
12	8.75	7.18	7.88	-0.12	22.00	14.85	17.29	+1.29	16.95	16.45	16.68	+0.68
13	9.71	8.14	8.70	+0.70	17.75	17.40	17.56	+1.56	21.32	20.50	21.04	+5.04
14	7.85	7.65	7.75	-0.25	20.40	16.45	18.43	+2.43	16.68	16.24	16.46	+0.46
15	8.70	7.15	7.84	-0.16	16.56	14.65	15.93	-0.07	16.45	14.26	15.18	-0.82
16	7.84	7.18	7.56	-0.44	13.38	12.55	12.83	-3.17	17.47	17.15	17.31	+1.31
Sample 4 (8.00-16.00-16.00)												
1	8.06	7.37	7.81	-0.19	15.95	15.65	15.85	-0.15	16.80	15.72	16.30	+0.30
2	7.31	6.12	6.63	-1.37	16.08	15.35	15.73	-0.27	16.83	16.71	16.77	+0.77
3	8.70	6.86	7.61	-0.39	16.39	15.57	15.97	-0.03	17.10	15.36	16.46	+0.46
4	6.21	5.56	5.90	-2.10	16.70	16.05	16.40	+0.40	16.64	16.56	16.60	+0.60
5	7.18	6.97	7.07	-0.93	16.12	16.10	16.11	+0.11	17.29	16.72	17.05	+1.05
6	9.08	7.26	7.92	-0.08	16.58	15.73	16.16	+0.16	17.46	16.86	17.10	+1.10
7	8.70	7.75	8.31	+0.31	15.95	15.15	15.54	-0.46	16.03	14.78	15.41	-0.59
8	7.60	7.45	7.53	-0.47	17.00	16.38	16.64	+0.36	16.64	16.28	16.46	+0.46
9	7.75	6.00	6.90	-1.10	16.60	16.32	16.47	+0.47	16.58	15.98	16.28	+0.28
10	7.80	7.26	7.47	-0.53	16.30	16.00	16.20	+0.20	16.60	16.04	16.41	+0.41
11	8.62	8.15	8.49	+0.49	16.15	15.95	16.05	+0.05	16.84	16.03	16.46	+0.46
12	8.65	6.85	7.35	-0.65	17.64	16.43	17.01	+1.01	18.80	13.90	16.31	+0.31
13	10.35	9.36	9.90	+0.90	16.35	15.85	16.10	+0.10	16.29	14.42	15.44	-0.44
14	7.80	7.50	7.65	-0.35	16.85	16.00	16.43	+0.43	16.52	16.24	16.38	+0.38
15	8.41	7.55	8.05	-0.05	16.13	15.72	15.97	-0.03	16.92	16.39	16.63	+0.63
16	8.57	7.67	8.13	+0.13	16.66	16.65	16.66	+0.66	17.31	17.01	17.16	+1.16

TABLE 3.—Continued

COLLAR- OMATOR	TOTAL NITROGEN				TOTAL PHOSPHORIC ACID (P <sub>2</sub> O <sub>5</sub> )				POTASH			
	HIGH	LOW	MEAN	VARIATION OF MEAN FROM THEORY	HIGH	LOW	MEAN	VARIATION OF MEAN FROM THEORY	HIGH	LOW	MEAN	VARIATION OF MEAN FROM THEORY

Sample 5 (8.00-16.00-16.00)												
1	8.05	7.85	7.94	-0.06	16.75	16.65	16.68	+0.68	15.87	15.72	15.82	-0.18
2	7.55	7.03	7.35	-0.65	16.68	15.34	16.28	+0.28	15.63	15.63	15.63	-0.37
3	8.02	7.47	7.71	-0.29	17.03	16.31	16.75	+0.75	17.79	16.18	17.09	+1.09
4	7.97	7.81	7.90	-0.10	17.15	16.90	17.02	+1.02	14.58	14.42	14.51	-1.49
5	8.44	7.74	8.02	+0.02	17.52	17.45	17.49	+1.49	16.67	15.79	16.24	+0.24
6	7.86	7.14	7.40	-0.60	17.53	15.95	16.87	+0.87	14.84	13.68	14.18	-1.82
7	8.10	7.60	7.91	-0.09	16.70	15.05	15.99	-0.01	15.54	14.26	14.87	-1.13
8	8.04	7.93	7.99	-0.01	16.50	16.08	16.29	+0.29	15.88	15.04	15.46	-0.54
9	7.93	7.40	7.71	-0.29	16.80	16.55	16.68	+1.03	16.36	15.58	15.97	-0.03
10	7.70	7.62	7.73	-0.35	17.25	16.85	17.03	+1.03	15.52	15.44	15.48	-0.52
11	7.97	7.60	7.73	-0.27	15.80	14.90	15.42	-0.58	18.09	17.09	17.63	+1.63
12	8.10	7.35	7.73	-0.27	17.10	15.15	16.24	+0.58	17.10	12.90	14.81	-1.19
13	8.26	7.87	8.03	+0.03	16.10	13.90	14.98	-1.02	16.20	15.70	15.95	-0.05
14	8.40	8.20	8.30	+0.30	17.75	16.95	17.35	+1.35	16.20	15.72	15.96	-0.04
15	8.04	7.67	7.92	-0.08	16.30	15.56	15.93	-0.07	17.01	15.46	16.47	+0.47
16												

Sample 6 (8.00-16.00-16.00)												
1	8.10	8.04	8.07	+0.07	16.25	16.10	16.17	+0.17	16.27	16.16	16.23	+0.23
2	7.95	7.66	7.85	-0.15	16.34	16.12	16.21	+0.21	16.56	16.50	16.53	+0.53
3	8.03	7.26	7.73	-0.27	16.15	15.81	16.01	+0.01	16.64	16.00	16.35	+0.35
4	7.96	7.60	7.78	-0.22	16.30	16.00	16.13	+0.13	16.12	15.70	15.89	-0.11
5	7.91	7.69	7.81	-0.19	16.43	16.07	16.25	+0.25	16.52	16.13	16.29	+0.29
6	8.36	7.78	8.10	+0.10	16.30	15.23	15.98	-0.02	16.54	16.16	16.34	+0.34
7	8.00	7.70	7.85	-0.15	16.45	16.30	16.35	+0.35	15.95	15.83	15.91	-0.09
8	7.84	7.66	7.75	-0.25	16.60	16.59	16.60	+0.60	15.90	15.22	15.56	-0.44
9	7.70	7.28	7.58	-0.42	16.35	16.30	16.33	+0.33	16.20	15.84	16.02	+0.02
10	7.98	7.70	7.87	-0.13	16.17	16.10	16.14	+0.14	16.24	16.16	16.20	+0.20
11	8.15	7.95	7.96	+0.06	16.35	15.25	15.73	-0.27	16.26	16.00	16.13	+0.13
12	8.17	7.18	7.59	-0.41	17.32	16.54	16.81	+0.81	16.20	16.10	16.13	+0.13
13	8.11	7.89	8.01	+0.01	15.85	15.78	15.83	-0.17	14.58	14.37	14.48	-1.52
14	7.50	7.50	7.50	-0.50	16.30	16.20	16.25	+0.25	16.36	16.24	16.30	+0.30
15	8.15	7.91	8.04	+0.04	15.98	15.77	15.86	-0.14	16.26	14.45	15.22	-0.78
16	8.18	8.01	8.08	+0.08	16.13	16.08	16.11	+0.11	16.52	16.27	16.40	+0.40





ever, do not tend to segregate to the same extent as do those that are dry and are composed of components that vary in particle size. The results of

TABLE 4.—*Theoretical value, and the mean of all results reported by the collaborators for each sample*

SAMPLE NO.	TOTAL NITROGEN		TOTAL P <sub>2</sub> O <sub>5</sub>		POTASH	
	THEORETICAL	FOUND	THEORETICAL	FOUND	THEORETICAL	FOUND
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	4.20	4.29	8.71	9.24	4.20	3.96
2	4.20	4.29	8.71	8.99	4.20	4.43
3	8.00	7.79	16.00	16.12	16.00	16.75
4	8.00	7.72	16.00	16.19	16.00	16.47
5	8.00	7.81	16.00	16.56	16.00	15.70
6	8.00	7.87	16.00	16.16	16.00	16.00
7	8.00	7.82	16.00	16.20	16.00	16.02
8	8.00	7.86	16.00	16.32	16.00	15.85

this collaborative study show conclusively that while the degree of grinding prescribed in the official method for the preparation of samples for analysis may be sufficient for the analysis of fertilizer materials and of

TABLE 5.—*Standard deviation from the theoretical values of the results reported by the collaborators for each sample*

SAMPLE NO.	STANDARD DEVIATION FROM THEORETICAL VALUES		
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
1	0.16	0.79	0.69
2	0.14	0.42	0.26
3	0.47	2.07	1.54
4	1.00	0.58	0.93
5	0.34	0.94	1.25
6	0.27	0.41	0.57
7	0.34	1.04	0.62
8	0.27	0.50	0.61

mixtures that are moist and sticky, finer grinding is necessary if accurate results are to be obtained in the analysis of dry segregating mixtures.

#### LITERATURE CITED

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- (3) JONES, C. H., and ROBERTSON, B. F., *Ibid.*, 287–89 (1920).
- (4) ROSS, WILLIAM H., and RADER, JR., L. F., *Ibid.*, **23**, 234–42 (1940).
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## REPORT ON NITROGEN\*

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

In last year's report, *This Journal*, 23, 242 (1940), the Associate Referee suggested that a certain copper powder be tried out as a possible catalyst for the determination of total nitrogen. Some work was done with this catalyst but the preliminary results appeared to be generally irregular. During the past year a new catalyst was mentioned in the literature,<sup>1</sup> and it seemed to give greater promise than either the copper or the usual mercury catalyst for shortening the regular Kjeldahl digestion.

This new catalyst consists of a mixture of 10 grams of anhydrous dipotassium phosphate, 6 grams of ferric sulfate, and the regular quantity of mercury. Results obtained on a variety of materials by the official Kjeldahl-Gunning-Arnold method were accurately checked by the new catalyst. The time of digestion, with gas burners, was reduced from 2.5 hours to 30 minutes. This short period was explained on the basis that more surface was provided for oxidizing activity and for the formation of gas bubbles. No noxious fumes were produced, and there was no increase in the number of steps in the procedure.

Preliminary work comparing this new procedure with the official method for total nitrogen was tried out by the Associate Referee on a variety of materials, with the results shown in Table 1.

TABLE 1.—Comparison of total nitrogen results by the official method and by the rapid  $Fe_2(SO)_4$  method

SAMPLE	OFFICIAL METHOD	RAPID METHOD	DIFFERENCE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Agrinite	8.44	8.49	+0.05
Hoof Meal	14.27	14.23	-0.04
Cocoa Meal	3.14	3.18	+0.04
Tobacco Stems	1.00	1.08	+0.08
Milorganite	5.69	5.72	+0.03
Smirow	7.08	7.07	-0.01
Peanut Hull Meal	1.24	1.22	-0.02
Ground Cocoa Cake	3.06	3.09	+0.03
Sludge	1.78	1.87	+0.09
Dried Peat	2.93	3.02	+0.09
Tankage (Armour)	8.55	8.57	+0.02
Tankage (Hynite)	9.42	9.46	+0.04
Castor Pomace	4.88	4.89	+0.01
Soil (surface)	0.103	0.102	-0.001
Soil (sub)	0.056	0.051	-0.005

\* Journal Series Paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Soil Chemistry and Bacteriology.

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 396 (1940).

It is quite evident from the results (Table 1) that the two methods are comparable, the rapid method giving results that average 0.03 per cent higher than those obtained by the official method. In the oxidation of these various organic products by the official method, the process is completed at varying time intervals, depending on the intensity of the gas flame and the nature of the material. The appearance of the digest is usually the criterion for determining when this point is reached. In the rapid method, the gas flame is immediately turned on at full strength and the process completed in exactly 30 minutes, provided the flasks are rotated and shaken as in the regular process. With some samples by the official method, it is often difficult to determine when the digestion is completed, whereas by the new procedure there is a more intense and complete oxidation.

Since an appreciable amount of time is saved in the digestion process by the ferric sulfate-dipotassium phosphate procedure, this method would seem to warrant an exhaustive collaborative study. The cost of the reagent is slightly greater than that of sodium sulfate, but owing to the saving in time the method is more economical.

Last year considerable collaborative work was reported on the beaker method for the determination of water-insoluble nitrogen, and this method was adopted as official (first action). Because of the nature of the results previously obtained, further collaborative work on this method was deemed unnecessary.

Details as to the methods of standardizing solutions for nitrogen work and for the preparation of methyl-red indicator were accorded official rating (first action) last year, and are now ready for final adoption.

The Associate Referee was also assigned the task of investigating the official method for the determination of chlorine in fertilizers containing Cyanamid. The official method for the determination of chlorine in fertilizers is a volumetric procedure and consists of titrating the fertilizer extract with standard silver nitrate solution. Potassium chromate is used as the indicator. However, when large quantities of Cyanamid are present in the extracted solution, silver cyanamide is formed, which seriously obscures the end point. According to a Florida modification of the official method (unpublished), if the extracted solution is slightly acidified with nitric acid and then neutralized with an excess of calcium carbonate, the titration will proceed normally. The addition of nitric acid converts the nitrogen of the Cyanamid to urea, with the result that it no longer reacts with the silver.

Mixed fertilizers containing varying quantities of Cyanamid were sent to several collaborators to be analyzed for chlorine by the official method and by the Florida modification. The results are given in Table 2. The Shuey modification (unpublished), reported in the third column of the table, consists in adding 1 ml. of hydrogen peroxide (30 per cent) to the

water extract before titration. Those collaborators using this modification reported that the end point was sharper.

It will be noted from the table that where 240 pounds of Cyanamid was used in the fertilizer, the Florida modification was essential to the ob-

TABLE 2.—Chlorine content of fertilizer samples containing varying quantities of Cyanamid (per cent)

COLLABORATOR	OFFICIAL A.O.A.C.	FLORIDA MODIFICATION	SHUEY MODIFICATION	THEORETICAL VALUE
<i>Sample A—240 lbs. Cyanamid</i>				
Hart	3.80	3.90	3.80	
Long	3.90	3.82	3.80	
Shuey	3.60	3.95	3.60	
Prince	2.00*	3.95		
Average	3.33	3.91	3.73	3.96
<i>Sample B—140 lbs. Cyanamid</i>				
Hart	3.90	3.95	3.90	
Long	3.80	3.95	3.80	
Shuey	3.70	3.76		
Prince	3.85	4.10		
Average	3.81	3.94	3.85	4.18
<i>Sample C—90 lbs. Cyanamid</i>				
Hart	4.20	4.30	4.30	
Long	4.20	4.35	4.20	
Shuey	4.22	4.36	4.16	
Prince	4.20	4.20		
Average	4.21	4.30	4.22	4.28
<i>Sample D—40 lbs. Cyanamid</i>				
Shuey	4.44	4.40		
Prince	4.35	4.40		
Average	4.40	4.40		

\* End point very uncertain.

taining of accurate results. The Associate Referee could not obtain a satisfactory end point by the official method under the same conditions, but by using the Florida modification he obtained a distinct end point and results that corresponded closely to the theoretical chlorine value. In the sample of fertilizer containing 140 pounds of Cyanamid, the official

method gave results that were approximately 0.13 per cent below those obtained by the Florida modification. However, with the samples that contained 90 and 40 pounds of Cyanamid, respectively, the results by the official method were not significantly different from the results by the Florida modification. Since few fertilizer mixtures contain over 100 pounds of Cyanamid per ton, it appears to the Associate Referee that it is unnecessary to change the present official method for the determination of chlorine.

In addition to the above work, the Associate Referee was asked to look into the subject of moisture in fertilizers. More specifically, it was desired to know to what fertilizer compounds the toluene method would apply. Correspondence with the former associate referee on this subject and with W. H. Ross of the Bureau of Chemistry, U. S. Department of Agriculture, definitely indicated that the toluene method should be limited to organic ammoniates and such anhydrous materials as urea, calnitro, and ammonium nitrate and to the determination of combined free water and water of hydration in such hydrated materials as calcium nitrate and Calurea, but should not include mixtures. To quote Ross: "Fertilizer mixtures usually contain superphosphate, and the principal components of a cured superphosphate are monocalcium phosphate, which contains water of crystallization, and anhydrous calcium phosphate. In the curing of superphosphate, more or less of the moisture or free water initially present is changed into water of crystallization and an automatic drying of the material therefore takes place. The physical properties of a superphosphate depend in a large measure on its free-water content rather than on the water of crystallization, and it is the free water in which the manufacturer is interested. Therefore, the toluene method for determination of moisture will not apply to mixed fertilizers, if only free water is desired." Ross uses the method of Hill and Jacob, *This Journal*, 17, 487 (1934), on superphosphate mixtures. It consists in drying the sample three hours in a vacuum oven over concentrated sulfuric acid. The method gives the free water only, and not the sum of the free water and the water of crystallization.

Since the subject of water relations in fertilizers is quite a complicated one, it would seem desirable to appoint a special referee to cover this subject.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That the ferric sulfate dipotassium phosphate method for shortening the Kjeldahl digestion be studied.
- (2) That the beaker method for the determination of water-insoluble nitrogen, *This Journal*, 22, 268 (1939), be adopted as official (final action).

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 46 (1941).

(3) That in the Kjeldahl method for the determination of organic and ammoniacal nitrogen, *Methods of Analysis*, A.O.A.C., 1940, 23, 19 (a), after the heading "Standard hydrochloric acid," the following portion of a sentence be added: "Proceed as directed under 5 and 6, Appendix 1, or" (final action).

(4) That in the Kjeldahl method for the determination of organic and ammoniacal nitrogen, *Ibid.*, 23, 19 (b), after the heading, "Standard sulfuric acid," the following portion of a sentence be added: "Proceed as directed under Appendix 1, or" (final action).

(5) That in the Kjeldahl method for the determination of organic and ammoniacal nitrogen, *Ibid.*, 24, 19 (i), line 2, the phrase "dilute to 100 ml. with H<sub>2</sub>O" be deleted (final action).

(6) That the official method for the determination of chlorine in fertilizers still be applicable to fertilizers containing no more than 100 pounds of Cyanamid.

## REPORT ON MAGNESIUM AND MANGANESE IN FERTILIZERS\*

By JOHN B. SMITH, *Associate Referee*, and E. J. DESZYCK  
(Agricultural Experiment Station, Kingston, R. I.)

The study of methods for the determination of magnesium and manganese, *This Journal*, 23, 247 (1940), was continued. The progress that has been made can be ascribed very largely to the suggestions and assistance of many generous collaborators. False starts have been made, certain accomplishments have been superseded by better proposals, and economic changes have modified the objectives. If progress has been slow, it is hoped that the study has been held to practical requirements, and that the choices made will be justified by use of the methods recommended.

### COLLABORATIVE ANALYSES

The objectives were the justification of a volumetric method for acid-soluble magnesium, further study of methods for water-soluble and acid-ammonium-citrate-soluble magnesium, and the comparison of two volumetric methods and a colorimetric procedure for acid-soluble manganese.

Three fertilizer bases, described in Table 1, were made from ingredients taken from commercial stocks at hand, except the ammoniated superphosphate, which was kindly supplied by M. H. Lockwood, Eastern States Farmers Exchange. To these bases were added three types of magnesium compounds and a common commercial brand of manganese sulfate. Sea-water magnesium oxide, recovered from the bittern resulting from the solar evaporation of sea water, is the most concentrated commercial car-

\* Contribution No. 537 of this Station.

rier of magnesium. Analysis of that used in Sample 1 showed 91.33 per cent magnesium oxide and 2.30 per cent calcium oxide. Actomag is a commercial brand of selectively calcined dolomite, in which the magnesium carbonate is decomposed to leave magnesium oxide, without material decomposition of the calcium carbonate present. That used in this study contained magnesium equivalent to 28.24 per cent magnesium oxide.

Two dolomites were supplied by W. H. Ross. These have been described previously in reports of vegetative studies to determine the availability of the magnesium contained, *This Journal*, 22, 137, 142 (1939); 23, 373 (1940). Dolomite A is a composite of equal parts of six separates of dolomites from three quarries. Dolomite B is a similar composite of a more soluble stone from a single quarry.

In addition to the magnesium in the carriers, there are small quantities in the other ingredients. These were determined in separate samples containing the formula ingredients in the prescribed proportions but omitting the carriers. The results, including a small blank on the reagents, were calculated on the basis of the complete formulas. This magnesium was shown to be soluble in water and in the citrate solution used in the methods submitted. The proper corrections were made in calculating recoveries from the entire mixtures, and from single ingredients.

Two samples were prepared for manganese by adding a commercial brand of manganese sulfate to the same basal fertilizers used for magnesium. A third sample was the manganese sulfate alone, and the average of the results reported by the collaborators for this sample by the bismuthate method was used in calculating recoveries for the other mixtures. Traces of manganese in the other ingredients were insignificant. The following methods were used:

(1) Acid-soluble magnesium by the Bartlett-Tobey Method, as changed last year, and by the volumetric modification described under Method 2 in *This Journal*, 23, 247 (1940).

(2) Magnesium soluble in acid ammonium citrate. Method 3 in the report cited above.

(3) Water-soluble magnesium. The following method is used in routine fertilizer inspection work in Maine.

Weigh a 1 gram sample into a 500 ml. volumetric flask, add 350 ml. of water, and boil for 1 hour. Cool, make to mark, and mix. Filter, and pipet 200 ml. into a 400 ml. beaker. Add approximately 2 grams of  $\text{NH}_4\text{Cl}$ . Proceed as directed in Method 1 for acid-soluble  $\text{MgO}$ , but add only 15 ml. of saturated  $\text{NH}_4$  oxalate, acidify the filtrate from Ca oxalate with 2 ml. of  $\text{HCl}$ , and evaporate this filtrate to 100 ml. before adding sodium citrate and proceeding with the precipitation of  $\text{MgNH}_4\text{PO}_4$ .

(4) Acid-soluble manganese,  $\text{KIO}_4$  volumetric method. The method discussed in *This Journal*, 21, 291 (1938) and in subsequent reports and published in *Methods of Analysis*, A.O.A.C., 1940, 37, 56, was again changed in minor detail to read as follows:

#### REAGENTS

(a) *Potassium permanganate*.—0.0910 N, 2.876 grams of  $\text{KMnO}_4$  in 1 liter of solution. Standardize with Na oxalate.



(b) *Ferrous sulfate*.—0.0910 *N*, 25.3 grams of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and 25 ml. of  $\text{H}_2\text{SO}_4$  in 1 liter of solution. Standardize with the 0.0910 *N*  $\text{KMnO}_4$ .

(c) *Mercuric nitrate*.—10%. Dissolve 10 grams of  $\text{Hg}(\text{NO}_3)_2$  in 90 ml. of water and clear with a few drops of  $\text{HNO}_3$ .

Weigh 1 gram of sample into a beaker and add 30 ml. of  $\text{HNO}_3$  and 10 ml. of  $\text{HCl}$ . Cover the beaker with cover-glass supported above the rim and digest at the boiling point for 30 minutes, adding more of the acids to prevent evaporation to dryness. To the solution, or to an aliquot if more than 15 mg. of Mn is present, add 15 ml. of a mixture of acids consisting of 1 part of  $\text{H}_2\text{SO}_4$ , 7 parts of 85%  $\text{H}_3\text{PO}_4$ , and 7 parts of water, by volume, and evaporate to white fumes in the covered beaker. If organic residues remain at this point, add successive portions of 5 ml. of  $\text{HNO}_3$ , repeating the evaporation after each addition until the organic matter is destroyed. Dilute to a volume of 50–75 ml. with  $\text{H}_2\text{O}$ . Add 0.3 gram of  $\text{KIO}_4$ . Heat below the boiling point for 30 minutes. Dilute to 150 ml., cool, and add 25 ml. of the  $\text{Hg}(\text{NO}_3)_2$  solution. Stir thoroughly, and filter the precipitate of I salts on a pad of asbestos on a Gooch crucible, using suction. Wash with water until the washings are no longer pink. Immediately reduce the  $\text{KMnO}_4$  in the filtrate with an accurately measured volume of 0.0910 *N*  $\text{FeSO}_4$ , using a small excess. Titrate the excess  $\text{FeSO}_4$  with 0.0910 *N*  $\text{KMnO}_4$ . 1 ml. of 0.0910 *N*  $\text{FeSO}_4$  = 1 mg. of Mn.

(5) Acid-soluble manganese, bismuthate method.

### Bismuthate Method

#### REAGENTS

(a) *Sodium bismuthate powder*.—80%  $\text{NaBiO}_3$ , not more than 0.0005% of Mn, and not more than 0.002% of Cl.

(b) *Potassium permanganate*.—0.0910 *N*, 2.876 grams of  $\text{KMnO}_4$  in 1 liter of solution. 1 ml. contains 1 mg. of Mn. Standardize with Na oxalate.

(c) *Ferrous sulfate*.—0.0910 *N*, 25.3 grams of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 25 ml. of  $\text{H}_2\text{SO}_4$ , and 25 ml. of 85%  $\text{H}_3\text{PO}_4$  in 1 liter of solution. 1 ml. = 1 mg. of Mn. Standardize with 0.0910  $\text{KMnO}_4$  near the time of actual use. Place a measured portion approximately equivalent to the maximum quantity of Mn to be determined in an Erlenmeyer flask containing 200 ml of cold  $\text{H}_2\text{SO}_4$  (3+97), and titrate with the  $\text{KMnO}_4$  solution.

#### DETERMINATION

To a 1 gram sample in an Erlenmeyer flask (preferably 300 ml.), add 5–10 ml. of  $\text{HNO}_3$  and 7 ml. of  $\text{H}_2\text{SO}_4$ . Evaporate on a hot plate to white fumes. Add a few drops of  $\text{HNO}_3$ , again evaporate to white fumes, and repeat until the organic matter is destroyed. Cool. Add 100 ml. of water, 10 ml. of  $\text{HNO}_3$ , and just enough of the  $\text{NaBiO}_3$  to give the solution a strong permanganate color, or in the case of a small quantity of Mn, a slight excess of  $\text{NaBiO}_3$ . Boil gently 2–3 minutes. If the permanganate color or  $\text{MnO}_2$  disappears, cool somewhat, and repeat the bismuthate treatment. A permanent permanganate color or the persistence of  $\text{MnO}_2$  indicates a sufficient excess of bismuthate. Add a saturated solution of  $\text{NaHSO}_3$  dropwise while stirring until the Mn compounds are reduced and the solution clears. Avoid a large excess. Boil gently 2–3 minutes. Cool to room temperature and make to a volume of approximately 100 ml. If the solution contains less than 40 mg. of Mn, proceed with the determination. If more than 40 mg. of Mn is present, transfer to a 200 ml. volumetric flask, add 5 ml. of  $\text{H}_2\text{SO}_4$  and 10 ml. of  $\text{HNO}_3$ , cool, dilute to volume, and mix. Pipet an aliquot containing not more than 40 mg. of Mn into an Erlenmeyer flask and dilute to 100 ml. with a water solution containing 5 ml. of  $\text{H}_2\text{SO}_4$ , and 10 ml. of  $\text{HNO}_3$  in 100 ml.

Before continuing, prepare suction filters of asbestos washed with the  $\text{H}_2\text{SO}_4$  and then with water. (Glass filter tubes with perforated porcelain disks to support the asbestos and connected with a suction flask are satisfactory. The Mn solution must not come in contact with rubber.) From this point complete the determination without interruption. To the Mn solution at 20–30°C. add at least 0.25 g of  $\text{NaBiO}_3$  for each 10 mg. of Mn. (It may be measured by weight or by a volume of known approximate weight relationship.) Swirl the contents of the flask for 1 minute, add 100 ml. of water, and mix. Filter with suction through the prepared filters and wash with cold  $\text{H}_2\text{SO}_4$  (3+97) until the washings show no pink tint. Disconnect the suction flask and from a buret add the  $\text{FeSO}_4$  solution until the permanganate color disappears; then add at least 10% in excess with 1 ml. as a minimum excess. Titrate the excess  $\text{FeSO}_4$  with the  $\text{KMnO}_4$  solution to a faint pink. From the  $\text{KMnO}_4$

TABLE 1.—*Samples for collaborative analysis*

SAMPLE	INGREDIENTS	POUNDS	MgO				Mn per cent
			DOLomite	OXIDE	CALCINED DOLomite	OTHER INGREDIENTS*	
			per cent	per cent	per cent	per cent	
(1) 6-12-4	Ammoniated Superphosphate, 3.75-18	1340					
	Sulfate of Ammonia	260					
	Muriate of Potash	134					
	Nitrogenous Tankage	200					
(2) 4-12-4	Sea-water Magnesium Oxide, 91.33% MgO	66		3.01	—	0.25	3.26
	Ammoniated Superphosphate	1340					
	Sulfate of Ammonia	106					
	Muriate of Potash	134					
(3) 7-7-7	Nitrogenous Tankage	100					
	Dolomite A, 19.41% MgO	320	3.15	—	—	0.24	3.39
	Superphosphate, 20%	700					
	Uramon (urea)	50					
	Soybean Meal	200					
	Sulfate of Ammonia	420					
(4)	Nitrate of Soda	125					
	Muriate of Potash	235					
	Dolomite B, 16.92% MgO	200					
	Actomag (selectively calcined dolomite), 28.24% MgO	70	1.69	—	0.99	0.20	2.88
(5)	Sample 2, but substitute Techmangam, 24.64% Mn, for Dolomite A						3.94
(6)	Sample 3, but substitute Techmangam for Dolomite B						2.46
	Techmangam (commercial grade MnSO <sub>4</sub> )						24.64

\* Traces of MgO in ingredients not used as MgO carriers, determined in mixtures made according to the formulas above, omitting the principal magnesium compounds. Results are calculated on the basis of the complete formula. Analysis showed this MgO entirely soluble in water and in acid NH<sub>4</sub> citrate.

equivalent to the ml. of  $\text{FeSO}_4$  solution used, subtract the  $\text{KMnO}_4$  used in the back titration. From the difference calculate percentage of Mn in the sample.

(6) Colorimetric modification of Method 4, *This Journal*, 23, 247 (1940).

TABLE 2.—Collaborators' results for MgO (per cent)

COLLABORATORS	SAMPLES					
	1	2	3	1	2	3
<i>Official Method, Acid-Soluble MgO</i>						
Deszyck	3.20	3.39	2.78			
<i>Bartlett-Tobey Method, Acid-Soluble MgO</i>						
<i>Gravimetric</i>			<i>Volumetric</i>			
Butt-Hallman	3.22	3.41	2.82	3.19	3.43	2.85
Byers	3.50	3.54	3.04	3.54	3.40	3.04
Deszyck	3.20	3.49	2.78	3.31	3.62	2.91
Gary	3.33	3.60	2.91	3.46	3.72	2.93
Hooper	3.20	3.46	2.92	3.29	3.37	2.90
Perkins	3.20	3.39	2.81	—	—	—
Hord-Purdy	3.05	3.29	2.63	3.19	3.27	2.69
Kleiber	3.14	3.34	2.81	3.18	3.36	2.80
Koch	3.20	3.29	2.65	3.30	3.45	2.68
Rader	3.20	3.36	2.88	3.20	3.26	2.78
Samuel-Blair-Hosey	3.30	3.38	2.84	3.20	3.42	2.82
Shuey	—	—	—	3.14	3.47	2.89
Spelman	3.32	3.38	3.04	3.28	3.37	3.08
Struve	3.36	3.48	2.86	3.41	3.55	2.94
Average	3.25	3.42	2.85	3.28	3.44	2.87
Recovery MgO (%)	100	101	99	101	101	100
<i>Water-Soluble MgO, Maine Method</i>			<i>Citrate-Insoluble MgO</i>			
<i>1 g : 350 ml., boiled 1 hr.*</i>			<i>6% <math>\text{NH}_4</math>-citrate, pH 4</i>			
Butt-Hallman	2.75	0.58	0.92	0.13	2.25	0.90
Byers	2.83	0.60	—	0.50	2.20	—
Deszyck	2.78	0.53	0.77	0.08	1.95	0.55
Gary	2.54	0.26	0.52	0.08	1.66	0.44
Hooper	2.88	0.61	0.84	0.06	1.72	0.47
Hord-Purdy	2.73	0.40	0.82	0.18	1.99	0.75
Kleiber	2.64	0.35	0.68	0.00	2.02	0.77
Rader	2.60	0.40	0.76	0.02	1.77	0.51
Samuel-Blair-Hosey	—	—	—	0.40	2.22	0.80
Shuey	2.89	0.55	0.88	—	—	—
Spelman	—	—	—	0.23	2.37	0.83
Struve	2.80	0.57	0.94	0.04	2.31	0.82
Average	2.74	0.49	0.79	0.16	2.04	0.68
Soluble MgO recovered from carriers (%)	83	8	22	95	37	74

\* By leaching 1 gram charges with 200 ml. of water at room temperature Deszyck reported 2.21%, 0.34%, 0.76%, for Samples 1, 2, 3; with 250 ml. water, Rader found 2.34%, 0.09%, 0.72%. With 200 ml boiling water, Deszyck found 2.44%, 0.38%, 0.82%.

## COLLABORATORS

Collaborators submitting results are: H. C. Batton and R. C. Koch, Swift and Company Fertilizer Works; C. A. Butt and A. O. Hallman, Int. Agr. Corp.; E. J. Deszyck; W. Y. Gary, Florida Agricultural Dept.; E. T. Hord and L. W. Purdy, North Carolina Dept. of Agriculture; W. C. Jones, B. L. Samuel, W. J. Blair, and A. D. Hosey, Virginia Dept. of Agriculture and Immigration; W. L. Kleiber, American Agricultural Chemical Co.; R. G. Kreiling and C. R. Byers, Armour Fertilizer Works; J. W. Kuzmeski, L. V. Crowley, and A. F. Spelman, Massachusetts Agricultural Experiment Station; L. F. Rader, Jr., U. S. Department of Agriculture; P. McG. Shuey, Shuey and Co.; O. I. Struve, Eastern States Coop. Milling Corp.; E. R. Tobey, J. F. Hooper, and G. H. Perkins, Maine Agricultural Experiment Station.

The results submitted are shown in Table 2 and are discussed with other pertinent data under the appropriate headings.

## ACID-SOLUBLE MAGNESIA

The Bartlett-Tobey method, discussed in previous reports, has continued to produce good results. The samples were less difficult than those analyzed last year, for manganese salts were not included. As a result the averages of all analyses are within a few hundredths of those calculated from the formulas, and the average deviations from the mean for Samples 1, 2, and 3 are only 0.09, 0.08, and 0.09 per cent for the gravimetric modification, and 0.09, 0.10, and 0.07 for the volumetric method. Averages of all results show no significant difference between the gravimetric and volumetric methods, and the deviations of individual results from the means are approximately alike for both modifications; 70 per cent of the results are within 0.1 per cent of the mean. It is difficult to ascribe a cause for the deviations that fall outside that limit, since an analyst seldom reports results that are consistently high or low. In this laboratory, after several years' experience with the Bartlett-Tobey method, single results out of line with known values occur more frequently than they do with other routine methods. It is seldom safe to rely on a single result by this method, but this is also true for any other procedure that has been tried for the determination of magnesium. The most probable errors are the retention of magnesium by the calcium oxalate and the passage of calcium into the filtrate during washing of the oxalate precipitate.

The collaborators express approval of both the volumetric and gravimetric procedures. Jones, Kleiber, and Shuey prefer the volumetric method because it is more rapid; Rader considers it a little more rapid and a little more accurate than the gravimetric method, but Struve and Spelman find no saving in actual working time. Certainly a result can be obtained sooner by the volumetric method, because ignition is omitted, but the time actually consumed in titration may be greater than that required

for weighing crucibles and precipitates. Gary suggests that in the volumetric method the precipitate be filtered in a glass filter tube, which seems allowable. Butt saves time in the same method by precipitation of calcium oxalate in the same 500 ml. volumetric flask used in dissolving the sample, followed by filtration and taking an aliquot. This procedure obviates

TABLE 3.—*Collaborators' results for acid-soluble Mn (per cent)*

COLLABORATORS	SAMPLES					
	4	5	6	4	5	6
	<i>KIO<sub>4</sub>, Volumetric</i>			<i>Bismuthate, Volumetric</i>		
Batton	3.83	2.34	24.80	3.84	2.36	24.31
Byers	3.93	2.50	—	3.98	2.59	—
Deszyck	3.84	2.45	24.87	3.83	2.45	24.58
Gary	3.92	2.49	24.03	3.97	2.44	24.60
Hord-Purdy	3.85	2.53	23.47	—	—	—
Kleiber	4.14	2.51	25.06	4.09	2.45	24.62
Kuzmeski	4.03	2.52	24.67	4.10	2.44	24.77
Crowley	4.04	2.45	24.83	3.97	2.39	24.84
Rader	4.11	2.51	25.12	4.05	2.54	25.14
Samuel-Blair-Hosey	3.89	2.36	24.46	3.97	2.42	24.45
Shuey	3.97	2.25	24.10	—	—	—
Struve	4.05	2.45	24.38	4.00	2.44	24.45
Average	3.97	2.45	24.53	3.98	2.45	24.64*
Recovery Mn (%)	101	100	100	101	99	—
	<i>KIO<sub>4</sub>, Colorimetric</i>					
Batton	4.10	2.65	24.80			
Byers	3.92	2.70	—			
Deszyck	3.83	2.41	—			
Gary	4.11	2.45	24.46			
Hord-Purdy	3.88	2.34	—			
Kuzmeski	4.08	2.47	24.92			
Crowley	4.00	2.42	24.84			
Rader	4.09	2.48	25.31			
Samuel-Blair-Hosey	3.90	2.44	—			
Shuey	3.89	2.31	—			
Struve	4.11	2.54	—			
Average	3.99	2.47	24.87			
Recovery Mn (%)	101	100	101			

\* Most reliable value for manganese in the manganese sulfate used as an ingredient in Samples 4 and 5.

washing the oxalate precipitate, but unfortunately it is not adapted to the determination of calcium in the same aliquot used for magnesium.

Gary questions the direction for precipitation of magnesium ammonium phosphate, "Stir vigorously until precipitation is complete." Deszyck and Spelman join Gary in the observation that magnesium in dilute solutions often does not precipitate until the addition of ammonia before the

period of standing and that sometimes an overnight period is required. This is quite true, and appropriate changes will be suggested in the method. Only samples with a few tenths per cent magnesium will be affected, but the final precipitation for such samples must be given considerable time before the analyst decides that no precipitate will form.

There are obvious advantages in combining the determinations of calcium and magnesium to use a single aliquot from the same sample. The Associate Referee on Calcium in Fertilizers, Gordon Hart, and his collaborators have approved the suggestion and apparently find it feasible, but they consider that contamination with iron and aluminum is decreased by precipitation of calcium oxalate at pH 3.5-4, as recommended by Hoffman and Lundell.<sup>1</sup> This condition is probably more important in the gravimetric method for calcium than in the permanganate titration method favored by Hart. However, if desired, the change of indicators to give this pH could be made in the Bartlett-Tobey method for magnesium without loss of accuracy. By this modification, Deszyck found 3.30, 3.55 and 3.92 per cent magnesium oxide for Samples 1, 2, 3, good agreement with the averages calculated in Table 2. The Bartlett-Tobey method will be kept in its present status for collaborative work with the Associate Referee on Calcium and not recommended for final adoption this year. The Bartlett-Tobey method is designated as Method II in *Methods of Analysis*, A.O.A.C., 1940, 36, 53.

Deszyck analyzed the collaborative samples by the official method (Table 2), and Kleiber found similar values by the Shuey method discussed in the previous report.

#### WATER-SOLUBLE MAGNESIUM

The method for the determination of magnesium in water-soluble compounds, changed in minor details last year to agree with the Bartlett-Tobey method, *Methods of Analysis*, A.O.A.C., 1940, 36, 55, was not studied further. No adverse criticism was received, and the procedure is recommended for final adoption.

The determination of water-soluble magnesium in fertilizer mixtures is a part of the routine fertilizer inspection in Maine, and water-soluble magnesium compounds have been recommended for tobacco fertilizers by agronomists of other sections. Other agronomists have considered the cheaper dolomite sufficiently reactive for their needs. Beside the water-soluble carriers, soluble products are formed during the curing process by reactions of other fertilizer ingredients with dolomite, selectively calcined dolomite, and magnesium oxide, often in very substantial amounts. E. R. Tobey has sponsored a method for this fraction that has been used in Maine, in several commercial fertilizer laboratories, and elsewhere.

Because of the general interest in this method, it was submitted to the

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<sup>1</sup> Nat. Bur. Standards Research Paper R. P. 1095, 616.

collaborators, The results reported in Table 2 are sufficiently consistent to show that the method is workable, and they confirm the experience of those who have used the method in routine analysis. From the materials studied this year, it may be calculated that 83 per cent of sea-water magnesium oxide formed soluble compounds, possibly during the boiling with water, since the ingredients were mixed in a dry state, *This Journal*, 23, 404 (1940). Under similar conditions, only 8 per cent of the magnesium in dolomite A was recovered. Sample 3 contained two magnesium carriers, making accurate calculations impossible, but if the dolomite was 8 per cent decomposed, about 45 per cent of the magnesium in the calcined dolomite became water-soluble. This calculation is based on an assumption that cannot be verified. It is about the same as the recovery reported by MacIntire, Hardin, and Oldham<sup>2</sup> for mixtures of 10 per cent of a similar material with superphosphate, boiled for 30 minutes with either 75 or 175 ml. of water. When boiled with 75 ml. of water the addition of ammonium sulfate increased the recovery only to 51 per cent, but 63 per cent was recovered by boiling a 1 gram charge with only 10 ml. of water. Recoveries of 70–80 per cent have been reported by commercial mixers, both by the dry and by the wet processes (private communication).

Attention has frequently been called to the fact that the method of extraction affects the apparent solubility of the relatively insoluble oxides and carbonates of magnesium in fertilizers.<sup>3</sup> This is further demonstrated by Rader and Deszyck (footnote, Table 2). For Samples 2 and 3, involving dolomite and calcined dolomite, only small differences resulted from leaching charges with cold or hot water, and by boiling with water as suggested by Tobey, but the recoveries of magnesium in Sample 1, which contained sea-water magnesium oxide, increased materially as the method of extraction was made more drastic.

The question of the origin of the water-soluble compounds has caused considerable interest. The most probable products of the reactions of magnesium oxide or carbonate with other fertilizer constituents are monomagnesium phosphate, relatively water-soluble; dimagnesium phosphate; and magnesium ammonium phosphate, usually considered insoluble, but actually appreciably soluble in the boiling suspension used by the collaborators. Deszyck added dimagnesium phosphate and magnesium ammonium phosphate, reagent grade, separately to each of the three samples to increase the magnesia content by 0.5 per cent, and recovered these additions quantitatively by the boiling procedure.

Since these products appear upon leaching or boiling a sample of dry-mixed ingredients, most workers believe that the reactions are caused by the extraction process, and that the components are not present before the addition of water. If water is present, these reactions are initiated during

<sup>2</sup> *Ind. Eng. Chem.*, 30, 651 (1938).

<sup>3</sup> *This Journal*, 20, 252 (1937); 23, 404 (1940); *Ind. Eng. Chem.*, 30, 651 (1938); *Am. Fertilizer*, 91, No. 12, 5 (1939).

mixing and continue during storage, for although the water used for extraction may cause further changes, Whittaker, Rader, and Zahn<sup>4</sup> show that the water-soluble magnesium decreases with temperature and time for three types of mixtures with an initial moisture of 10 per cent. This is true whether the extraction is by leaching at room temperature or by boiling with water, *This Journal*, 23, 404 (1939). Struve (private communication) at two week intervals, analyzed two commercial mixtures containing magnesium oxide, triple superphosphate, sulfate of ammonia, a nitrogen solution, muriate of potash, and an organic conditioner. Results were approximately the same for two grades, 7-14-21 and 8-16-16. Moisture results were lower than those in the work cited above, and they decreased during storage from approximately 5 per cent at the start to about 2 per cent, as determined by oven-drying. Free water is changed rapidly to water of hydration in the initial stage of these reactions, and it may have decreased somewhat even before the first determination; but apparently the small quantity of water reported was sufficient to cause changes, for by the boiling process, water-soluble magnesium increased significantly for 6 weeks and then became about constant for the remainder of the 10-week period. Obviously the extraction process is not the only factor causing the reactions, or results would not vary with time of storage, but the evidence indicates that it has an effect.

Variability of results by the different methods of extraction must be considered in relation to the objectives of the method, and here there is a divergence of opinions. Although the cost of the products that are more reactive than dolomite has decreased, they are more expensive than the limestone, and some fertilizer manufacturers desire to obtain a method that will discriminate in favor of such products. Some agronomists believe that such discrimination is unfair to the actual usefulness of dolomite. Some chemists believe that analyses in the control laboratory should describe the components of a fertilizer as it is sold, while others think that these analyses should reflect the reactions anticipated under average cropping conditions. It is difficult to reconcile these views. Certainly the determination of the water-soluble fraction of magnesium is the most discriminatory suggestion that has been made, and more time for fact-finding and better agreement among interested parties is needed in order to arrive at a fair decision.

#### ACTIVE OR AVAILABLE MAGNESIUM

The correlation of the solubility of magnesium compounds in acid ammonium citrate with the usefulness of these compounds as fertilizers was discussed in considerable detail in *This Journal*, 23, 247 (1940). The method specifying that solvent is intended to include the sulfates, nitrates, and chlorides, all the usual phosphates, the oxides, and as much of the

<sup>4</sup> *Am. Fertilizer*, 91, No. 12, 5 (1939).



natural carbonates in dolomite as is likely to decompose in the average soil at pH 5.5 during a cropping season of 2-3 months. The rate of decomposition is known to vary with particle size, crystal structure or composition, acidity, and buffer capacity of the soil, and doubtless there are other factors. Therefore, this method is open to many of the criticisms brought against the method for water-soluble magnesium in the previous section of this report. A redeeming feature of the method may be the fact that it recovers a conservatively chosen portion of dolomitic magnesium and is therefore less discriminating against dolomite than is water extraction.

Briefly, the method is similar to the official method for the determina-

TABLE 4.—Rate of decomposition of dolomite in soil and the solubility of separates calculated from areas of particles and of three dolomites in acid ammonium citrate

MESH	DECOMPOSITION IN SOIL AFTER				SOLUBILITY OF MgO IN		SOLUBILITY OF MgO IN	
	2-3 MONTHS				DOLOMITE WITH		DOLOMITE ALONE	
	1 pH 5-5.5	2 DOLOMITE*	3 pH 4.6- 5.6		4 6-8-6 FERTILIZER*	5	0.2 g. CHARGE 6	1 g. CHARGE 7
	per cent	per cent	A	per cent	per cent	per cent	per cent	per cent
20-40	15	16	A	23	21	27	41	31
40-60	25	28	A	41	33	43	51	47
60-80	50	40	A	53	41	51	71	49
80-100	50	(50)	A	60	50	56	70	52
100-200	60	61	A	74	52	62	76	54
Through 200	75	90	A	85	78	79	93	68
Composite	50	—	A	54	46	52	68	46
Composite	50	—	B	61	64	65	74	61
Composite	50	—	C	52	26	28	52	29

\* Supplied by W. H. Ross for collaborative vegetation and chemical studies.

(1) General averages from results by 5 groups of workers, Smith, *This Journal*, 23, 247 (1940).

(2) Calculated by C. J. Schollenberger from particle area, assuming 50% decomposition of 80-100-mesh separate, Column 1.

(3) Averages of recent work by two groups of workers, Rader, Zahn, and Whittaker, *This Journal*, 23, 404 (1940).

(4), (6) Smith, *loc. cit.*

(5), (7) Rader, et al., *loc. cit.*

tion of available phosphoric acid, except that the neutral ammonium citrate solution is replaced by a solution made by adjusting a solution containing 60 grams of citric acid to pH 4 with ammonium hydroxide and finally making to a volume of 1 liter, maintaining the acidity at pH 4.

Data to substantiate the method are shown in Table 4. The first column shows an estimate of the rate of decomposition of dolomite for rather restricted conditions, made by the Associate Referee and based on the available published research. The values in the second column, calculated by C. J. Schollenberger from a formula developed at the Ohio Agricultural Experiment Station, are based on the reduction in diameter of particles in the various separates as concentric shells are dissolved from the particles.<sup>5</sup> The calculations, based on 50 per cent decomposition of 80-

<sup>5</sup> *Ind. Eng. Chem.*, 24, 998 (1932); Symposium on lime, Amer. Soc. Testing Materials, Philadelphia, 1940, and private communication.

100-mesh dolomite as listed in Table 4, show that if that value is correct, the percentage decomposition assigned to the other separates corresponds closely with the calculations, except for a lower estimate of particles passing 200 mesh as compared with the calculated value. For this size class the estimate in Column 1 might justifiably have been increased from the data, for the lower value of 75 per cent is a conservative choice.

The remaining columns show the results of work with dolomites and a fertilizer supplied by W. H. Ross for collaborative vegetation and soil tests and for chemical studies. These samples are intended to represent the common types of commercial dolomites. Column 3 shows the average decomposition of the limestone in soils, some of which are more acid than those included in Column 1, based on the determination of residual carbonates. These values seem to show more decomposition than those published by the Associate Referee, but the differences are not large.

Columns 4 and 5 show the percentages of the various materials dissolved by the acid ammonium citrate solution in the presence of the 6-8-6 fertilizer. These determinations, from two different laboratories, do not differ greatly, and in several instances they are consistent with the soil data. Certainly the effect of particle size is reflected, and the greater reactivity of Composite B as compared with Composite A is shown by both solution and soil. Dolomite C is thought to be less easily decomposed than Dolomite A. In this laboratory a much greater concentration of hydrochloric acid was required to recover fresh applications of Dolomite C in soil by the method for residual carbonates than was needed for Dolomites A and B, or was reported to have been used in the determinations from pot tests. Possibly this may explain the discrepancy between the values of Composite C in soil and solution (Columns 3, 4, 5). In Column 1 the general figure of 50 per cent was ascribed to all three composites, without differentiation.

Columns 6 and 7 present an interesting detail of the method. Beeson and Ross<sup>6</sup> have shown that the weight of charge as well as particle size is a factor in the solubility of dolomite in neutral ammonium citrate solution. For that reason, 0.2 gram was chosen for the work reported in Column 6, approximately equivalent to 4 per cent of magnesium in a gram charge. The solubility of dolomite alone was decidedly greater than when it was mixed with the water-insoluble residues of fertilizers. Rader et al., as noted in the table, increased the charge to 1 gram and found lower results in much better agreement with the solubility as a fertilizer ingredient. Whittaker, Rader, and Zahn, *This Journal*, 22, 180 (1939), tried the solubility of magnesium in mixtures of equal weights of a dolomite with three phosphates: monocalcium phosphate, double superphosphate, and superphosphate. For double superphosphate and monocalcium phosphate there was little difference, but in the presence of superphosphate the solubility

<sup>6</sup> *Ind. Eng. Chem.*, 29, 1176 (1937).

decreased. For three collaborative samples reported on last year, a 12-16-12 containing diammonium phosphate and double superphosphate, a 4-12-4, and a 0-12-0 with superphosphate, recoveries of magnesia from the same dolomite by the citrate solution were 66, 55, and 50 per cent, respectively. Recovery of magnesia from Dolomite A with a 6-8-6 fertilizer averaged 49 per cent (Table 3), but from a 4-12-4 the collaborators this year recovered 37 per cent (Table 2). Since all water-soluble ingredients are leached from the sample before the citrate solution is used, these observations suggest that calcium phosphate and possibly calcium sulfate in the residues influence the action of the solvent.

In an attempt to separate the factors, the data in Table 5 were obtained. In one series the calcium was increased, and in the other, the phosphate, without any change in the pH of the solutions. Small charges

TABLE 5.—*Effect of calcium and phosphate on solubility of dolomite in the acid ammonium citrate solution*

CaO EQUIVALENT ADDED TO SOLVENT IN EXCESS OF THAT IN THE DOLOMITE CHARGE	RECOVERY FROM DOLOMITE		P <sub>2</sub> O <sub>5</sub> EQUIVALENT ADDED TO SOLVENT	RECOVERY FROM DOLOMITE	
	MgO	CaO		MgO	CaO
<i>mg./100 ml.</i>	<i>per cent</i>	<i>per cent</i>	<i>mg./100 ml.</i>	<i>per cent</i>	<i>per cent</i>
0	70	70	0	70	70
50	54	57	100	61	61
100	50	53	200	66	68
150	43	47	300	63	64

Acid-ammonium-citrate solution modified by addition of Ca(NO<sub>3</sub>)<sub>2</sub> or (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> to citric acid solution before adjustment to pH 4 with NH<sub>4</sub>OH; 0.2 gram charges of Dolomite A (19.32% MgO, 28.02% CaO, 80-100 mesh) extracted for 1 hour at 65° C. as directed in the method proposed for fertilizers.

of dolomite were placed in these solutions and subjected to the usual treatment prescribed for the acid ammonium citrate solution. Clearly, calcium had the greater and more consistent influence in decreasing the rate of dissolution of the dolomite. Furthermore, the calcium and magnesium were removed from the particles in equal proportions, indicating that a factor that depressed the solubility of one would have a like effect on the other. This is consistent with a low solubility product of calcium citrate in the citrate solution. It is known that calcium citrate is much less soluble than the magnesium salt, and it is possible that relatively low concentrations of calcium decrease the rate of solubility, although larger amounts of calcium may be in solution when equilibrium is reached. This question needs more study.

Rader et al., *This Journal*, 23, 404 (1940), found approximately complete solubility of magnesium oxide in the citrate solution, when mixed with superphosphate, ammoniated superphosphate, or a 6-8-6 fertilizer, in proportions ranging from 40 to 160 pounds per ton with the phosphates, and 40 to 80 pounds with the fertilizer. MacIntire et al.<sup>2</sup> report complete

removal of the magnesia in selectively calcined dolomite with superphosphate and ammoniated superphosphates by neutral ammonium citrate solution, and there can be no doubt that the magnesia is equally soluble in the acid citrate.

In the previous report, six collaborators obtained nearly uniform results for three samples. Although the samples sent out this year are not greatly different from those of last year, the results for this method in Table 2 are much less consistent. Sample 1 with magnesium oxide caused no great difficulty, for only 2 of 11 results are out of line, and practically all the magnesium in the sample was soluble. Sample 2 had 3.15 per cent of magnesia from Dolomite A and 0.24 per cent from other ingredients. From the average acid-soluble magnesia reported in Table 2, the citrate-insoluble magnesia, and the fact that the magnesium from "other ingredients" is citrate soluble, recovery from the dolomite may be calculated. The average recovery is 37 per cent, but the results are rather evenly distributed between the lowest recovery, 26 per cent, and the highest, 49 per cent. To calculate the solubility of Dolomite B in Sample 3 it is necessary to assume complete solubility for the calcined dolomite. Then recoveries of magnesia from this more soluble dolomite range from 46 to 73 per cent, with the average of 59 per cent, and again there is an even distribution of results between the lowest and highest. There was a distinct tendency for some laboratories to report low results on Samples 2 and 3, while others secured high results.

As has been stated, the discrepancies among analysts were not anticipated from the results for last year and from experiences in this laboratory, but they are too great to justify recommendation of the method at present. The work will be continued, and criticisms and suggestions are solicited.

#### MANGANESE

The study of the volumetric and colorimetric methods based on oxidation with potassium periodate was continued from last year. At the suggestion of W. Y. Gary, a modification of the bismuthate volumetric method was added. Sodium bismuthate as an oxidizing agent was tried previously, *This Journal*, 21, 277 (1938), and discarded because of an unstable end point and the better adaptation of periodate to the colorimetric method. At that time the double treatment with bismuthate usually specified was omitted, as suggested by Collins and Foster.<sup>7</sup> The method advocated by Gary retains this double treatment with bismuthate to assure the oxidation of remaining traces of chlorides, organic compounds, or other slowly oxidized material. Because of this modification or other improvements, the method proved very satisfactory in its present form. Both volumetric methods require about equal amounts of time, but bis-

<sup>7</sup> *Ind. Eng. Chem.* 16, 586 (1924).

muthate may be used with larger aliquots than periodate without difficulty in filtration. Relatively large quantities of manganese may be oxidized by potassium periodate, but the iodine salts must be precipitated with mercuric nitrate, and the larger quantities of periodate required form a precipitate that is very slow to filter through asbestos. Filtration through special crucibles with sintered glass bottoms is more rapid, but even with this special apparatus the method appears to have little advantage over the bismuthate method. For the colorimetric method, the periodate is much superior for no filtration of the excess oxidizing agent is required.

All collaborators reported excellent results (Table 3) for Samples 1 and 2 by the three methods, allowing choice to be made on the basis of ease and celerity of manipulation. Sample 3 is a commercial manganese sulfate with a much larger percentage of manganese. The differences among analysts are greater for this method, and the bismuthate method shows the best uniformity.

The periodate method frequently allows manganese dioxide to form. This error is easily detected in the ocular colorimeter, but may escape notice in a photoelectric colorimeter or in the volumetric modification, and its occurrence is unpredictable. Kuzmeski suggests, and Deszyck confirms the conclusion, that the dioxide results from reduction of potassium permanganate by the unoxidized manganous ion during the course of oxidation. By reversing the procedure, pipetting the aliquot containing the manganous ion into a solution containing the periodate, no dioxide was noted. Also, it seems better to oxidize at the boiling point rather than to start the reaction at room temperature.

Although the colorimetric method is not considered adaptable to high concentrations of manganese because of a large dilution factor, a few analysts tried it with better success than was anticipated. Again Kuzmeski suggests that a light filter with either the ocular or photoelectric colorimeter allows the use of a standard solution containing 100 p.p.m. of manganese and greatly extends the range of concentrations that may be used, and he presents data to establish the fact. This point will be considered in the future. He also believes that oxidation of organic matter is best accomplished by boiling the sample to fumes with sulfuric acid and adding nitric acid drop by drop to the hot solution.

Gary, Batton, Struve, Jones, and Kleiber prefer the bismuthate method to the periodate method. Shuey likes the periodate volumetric method and Jones, Rader, Deszyck, and Kuzmeski consider the colorimetric method the best where it is applicable. Batton and Gary filter the precipitate from the iodine salts in the volumetric periodate method through asbestos in a glass filter tube, while Struve prefers a small Büchner funnel to the Gooch crucible. Batton warns against traces of paraffin from acid bottles. Shuey prefers ferrous ammonium sulfate to ferrous sulfate as a

reducing agent, and all the collaborators agree that the ferrous sulfate solution must be restandardized frequently. Jones suggests a modification to allow manganese to be determined in an aliquot from the phosphate determination. Apparently the popular choice is the bismuthate method, supplemented by the colorimetric periodate method for laboratories that have the equipment.

#### RATE OF DECOMPOSITION OF MAGNESIUM OXIDE IN SOIL

In a previous section it was shown that the determination of the magnesium actually remaining in fertilizers as the oxide may be a difficult task because of the effect of water on the reactions with other ingredients. If, however, magnesium oxide is as reactive in soil as its chemical nature would suggest, the formation of water-soluble compounds in the laboratory extraction may not go far enough to compare with changes in the soil. The task here is to measure the reaction of as small quantities of the material as is practicable, without using more water than is normally present in soils. The first attempt, based on displacement of the soil solution after adding magnesium sulfate or magnesium oxide, failed because base exchange reactions or capillary pores retained a large fraction of the magnesium added in either form.

Another line of attack was adopted, essentially a potentiometric titration of the soil at a normal state of moisture with dry magnesium oxide compared with dry calcium hydroxide. Three soils were chosen. Two were very fine sandy loams from the experimental plats of this Station, one of which had been limed and fertilized, while the other had received neither lime nor fertilizer. Each had an exchange capacity of about 11 milli-equivalents. The third soil was a very light sandy loam, of low but undetermined exchange capacity. The soils were air-dried, and lots equivalent to 200 grams of soil with 20 per cent moisture on the moist-soil basis were weighed. Sea-water magnesium oxide and reagent-grade calcium hydroxide were added to separate lots of the soils at the rate of from less than 2 to about 9 milli-equivalents for 100 grams of moist soil, and the samples were mixed very thoroughly. Measured quantities of water were then sprinkled over the soil to make 20 per cent of moisture on the moist-soil basis, and the samples were remixed and placed in covered glass jars. Other portions were prepared without added base. The samples were remixed daily for a week. At the end of that period the *pH* was determined by packing the soil about the electrodes of a Beckman glass electrode potentiometer without further additions of water. Readings were stable and reproducible, and the only precaution necessary was to make sure that the electrode surfaces were moist before the soil was placed about them. The results were verified by repetition of the experiment.

The results are shown in Figure 1. Apparently the reactions reached equilibrium, since measurements after 2 and after 3 weeks showed no

further change in pH. Also, 5 milli-equivalents of magnesium oxide after a week produced the same pH in the most acid of the three soils when maintained at 20, 25, 30, 35, and 40 per cent moisture for a week. On an equivalent basis the effects of magnesium oxide and calcium hydroxide were equal. As was anticipated, the bases were relatively more effective

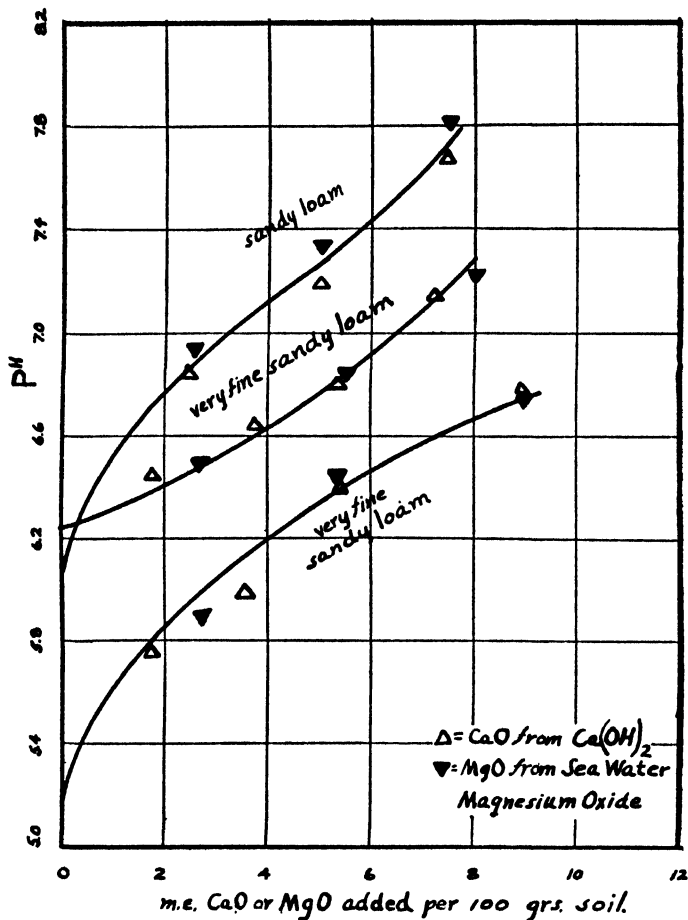


FIG. 1.—EFFECT OF MAGNESIUM OXIDE AND OF CALCIUM HYDROXIDE ON THE pH OF SOIL WITH 20 PER CENT MOISTURE AFTER 7 DAYS. THE DETERMINATIONS WERE MADE BY PACKING THE SOIL ABOUT A GLASS ELECTRODE WITHOUT INCREASING THE MOISTURE.

in the most acid soil, but there was a decided pH change in soils initially at pH 6, both in the moderately buffered very fine sandy loam and in the poorly buffered sandy loam. If the relatively large additions used in this experiment react to such an extent within a week, it appears certain that the smaller quantities applied as fertilizer ingredients would rapidly become available for adsorption by plants, or by the colloidal soil complex.

## RECOMMENDATIONS\*

It is recommended—

(1) That the method, entitled "Magnesium in water-soluble compounds," *This Journal*, 21, 77 (1938) and modified in minor detail, *This Journal* 23, 247 (1940), at present adopted as official (first action), *Methods of Analysis*, A.O.A.C., 1940, 36, 55, be adopted as official (final action).

(2) That the Bartlett-Tobey method for acid-soluble magnesium, *This Journal*, 22, 270 (1939), as modified last year, *This Journal* 23, 247 (1940); *Methods of Analysis*, A.O.A.C., 1940, 36, 53, be further changed by substituting for the sentence, "Stir vigorously until precipitation is complete," the sentence, "Stir vigorously until a precipitate forms," and adding after the words "or allow to stand overnight," the sentence, "if only very small quantities of magnesium are present, and no precipitate forms during stirring, or after adding 15 ml. of  $\text{NH}_4\text{OH}$ , the precipitate may appear during the overnight period."

(3) That the volumetric modification of the Bartlett-Tobey method for acid-soluble magnesium, *This Journal*, 23, 249 (1940), as changed by Recommendation 3 of that report, and adopted as a tentative method last year, *Methods of Analysis*, A.O.A.C., 1940, 36, 54, be adopted as official (first action).

(4) That the study of methods for acid-soluble, water-soluble, and active magnesium in fertilizers be continued.

(5) That the colorimetric method for acid-soluble manganese, *This Journal*, 23, 262 (1940), adopted as a tentative method last year, *Methods of Analysis*, A.O.A.C., 1940, 37, 58, be adopted as official (first action) and that it be entitled "(Applicable to samples with not more than 5 per cent manganese)".

(6) That the bismuthate method for acid-soluble manganese, published as Method 5 of this report and in *This Journal*, 24, 69 (1941), be adopted as tentative.

(7) That the study of methods for acid-soluble manganese, including the potassium periodate volumetric method, be continued.

## REPORT ON POTASH

By O. W. FORD (Purdue University Agricultural Experiment Station,  
West Lafayette, Ind.), *Associate Referee*

In accordance with the recommendations of the Association, *This Journal*, 23, 51 (1940), referee work was conducted this year by collaboration.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 46 (1941).



**PRESENT OFFICIAL METHOD FOR POTASH (METHOD A) *VERSUS*  
DISSOLVING OUT THE POTASSIUM CHLOROPLATINATE AND  
REWEIGHING (METHOD B)**

Four samples of fertilizer, numbered MF 115, 116, 117, and 119 were sent to 12 chemists with the following instructions:

Prepare sufficiently large composite solutions from samples MF 115, 116, 117, and 119 for six potash determinations as follows:

A. Make six determinations of potash on each sample by the present official method.

B. Make six determinations of potash on each sample by dissolving out the  $K_2PtCl_6$  and reweighing.

1. List the six individual potash determinations of each sample by Methods A and B.

2. Indicate whether the ignitions were made in silica or platinum dishes.

3. State the approximate final temperature of ignition in degrees centigrade, indicating the source of heat.

4. Name the type of filter, whether asbestos-padded Gooch or of sintered glass; if the latter, list the number and porosity.

Total number of potash determinations, 48.

*per cent*

$K_2O$  value of Samples MF 115— 8.0

MF 116—11.0

MF 117—17.0

MF 119—11.0

The results of 10 of the 12 collaborating chemists that reported are given in Table 1. The average results obtained by Method A are approximately 0.2 per cent higher than those obtained by Method B. One chemist found no difference between the results from Methods A and B. One other chemist obtained a higher value on one sample by Method B than he did by Method A. With these two exceptions each chemist reported a higher value for each sample by Method A than he did by Method B. Sufficient material was added to each of the four samples to give about 0.20 per cent insoluble residue, even with the best ignition. In a few cases higher residues were reported, but these were to be expected when the existing variable conditions of the different laboratories were taken into consideration. Most of the differences can be accounted for after a study of the comments of the collaborators in connection with their results (Table 1).

Considered as a whole the results for the various samples are quite consistent; they indicate that in Method A, the present official method, the directions should be modified to include the removal of the water-insoluble residues. This can be done with the least added effort and expense of time by dissolving out the potassium chloroplatinate and reweighing. If glass sinters are used for the filtration in dissolving out the potassium chloroplatinate, time can be saved if they are placed in a flat-bottomed container (a low-form Pyrex baking dish has been used to advantage), covered with hot water, and finally washed on suction with hot water and

TABLE 1.—*Collaborative results on potash*

ANALYST ANALYSES		METHOD A			METHOD B			DIFFERENCE IN K <sub>2</sub> O (%) A-B
NO.	NO.	HIGH	LOW	AV.	HIGH	LOW	AV.	
K <sub>2</sub> O Sample MF 115 (Method A) <i>versus</i> Dissolving out K <sub>2</sub> PtCl <sub>6</sub> and Reweighing (Method B)								
1	6	8.25	8.02	8.11	8.13	8.02	8.06	-0.05
9	6	8.50	8.37	8.42	8.37	8.27	8.34	-0.08
11	6	8.32	8.21	8.28	8.07	8.05	8.06	-0.22
13	6	8.32	8.24	8.29	8.12	7.96	8.08	-0.21
15	6	8.20	8.02	8.09	8.08	7.93	8.01	-0.08
16	6	8.50	8.45	8.48	8.50	8.45	8.48	0.00*
19	6	8.56	8.30	8.36	8.32	8.05	8.12	-0.24
20	6	8.56	8.31	8.47	8.29	8.12	8.22	-0.25
22	2	8.78	8.69	8.74	8.14	8.09	8.12	-0.62
24	4	8.37	8.22	8.29	8.08	7.96	8.02	-0.27
Average		8.44	8.28	8.35	8.21	8.09	8.15	-0.22
Max. variation		0.53	0.67	0.65	0.43	0.52	0.47	0.57
K <sub>2</sub> O Sample MF 116 (Method A) <i>versus</i> Dissolving out K <sub>2</sub> PtCl <sub>6</sub> and Reweighing (Method B)								
1	6	11.29	11.19	11.22	11.24	11.12	11.18	-0.04
9	6	10.89	10.81	10.84	10.79	10.68	10.74	-0.10
11	6	10.91	10.87	10.90	10.78	10.75	10.77	-0.13
13	6	11.76	11.64	11.71	11.60	11.48	11.55	-0.16
15	6	11.07	10.80	10.95	11.01	10.70	10.87	-0.08
16	6	11.22	11.15	11.19	11.22	11.15	11.19	0.00*
19	6	10.88	10.73	10.81	10.69	10.53	10.62	-0.19
20	6	11.08	10.93	11.03	10.97	10.85	10.91	-0.12
22	2	11.77	11.63	11.70	10.51	10.50	10.51	-1.19
24	4	11.52	11.40	11.45	11.24	11.14	11.20	-0.25
Average		11.24	11.12	11.18	11.01	10.89	10.95	-0.25
Max. variation		0.89	0.91	0.90	0.91	0.95	1.04	1.15
K <sub>2</sub> O Sample MF 117 (Method A) <i>versus</i> Dissolving out K <sub>2</sub> PtCl <sub>6</sub> and Reweighing (Method B)								
1	6	18.08	17.99	18.02	18.16	18.01	18.10	+0.08
9	6	17.93	17.72	17.85	17.76	17.60	17.70	-0.15
11	6	17.73	17.65	17.69	17.54	17.51	17.52	-0.17
13	6	18.24	17.92	18.05	18.08	17.68	17.85	-0.20
15	6	17.69	17.54	17.65	17.63	17.52	17.57	-0.08
16	6	18.55	18.45	18.51	18.55	18.45	18.51	0.00*
19	6	17.87	17.64	17.65	17.64	17.38	17.47	-0.18
20	6	17.98	17.83	17.92	17.75	17.63	17.69	-0.23
22	2	18.56	18.56	18.56	17.84	17.80	17.82	-0.74
24	4	17.87	17.76	17.83	17.58	17.42	17.51	-0.32
Average		18.05	17.91	17.97	17.85	17.70	17.77	-0.21
Max. variation		0.87	1.02	0.91	1.01	1.03	1.00	0.82
K <sub>2</sub> O Sample MF 119 (Method A) <i>versus</i> Dissolving out K <sub>2</sub> PtCl <sub>6</sub> and Reweighing (Method B)								
1	6	11.59	11.15	11.41	11.33	11.02	11.12	-0.29
9	6	12.02	11.86	11.93	11.92	11.73	11.84	-0.09
11	6	11.78	11.71	11.74	11.57	11.54	11.56	-0.18
13	6	12.08	11.92	12.02	11.76	11.68	11.73	-0.29
15	6	11.63	11.40	11.48	11.51	11.34	11.41	-0.07
16	6	12.10	12.05	12.07	12.10	12.05	12.07	0.00*
19	12	11.89	11.58	11.69	11.80	11.47	11.61	-0.08
20	6	11.86	11.74	11.79	11.86	11.72	11.77	-0.02
22	2	12.40	12.33	12.37	11.81	11.78	11.80	-0.57
24	4	11.74	11.61	11.69	11.58	11.46	11.50	-0.19
Average		11.91	11.74	11.82	11.72	11.58	11.64	-0.20
Max. variation		0.77	1.18	0.96	0.59	1.03	0.95	0.55

\* Omitted from average of column.

alcohol. One advantage of the glass sinter over the asbestos padded Gooch is that no material is removed unless it is water-soluble; it is also not only easy to see when all the potassium chloroplatinate has been dissolved but likewise, whether any insoluble residue has been picked up during the determination. Many chemists have called the attention of the Associate Referee to the fact that the present official method often gives too high results. One chemist in particular (13) has so expressed himself and asked the writer to try out his modification of the present official method on a few of the referee samples. His modification relates to the preparation of the solution for analysis and is essentially as follows:

Wash the sample on paper 2-3 times with hot water, once with hot saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$ , and again several times with hot water. Add  $\text{NH}_4\text{OH}$ , make to volume, filter, and proceed as directed in the official methods for potash.

Results by this method reported by Analyst 13 and the writer show lower potash values than do those obtained by the present official method. Analyst 13 did not report insoluble residue by the modification, but the Associate Referee found water-insoluble residues, although not in so large amounts as shown by the official method. These results are what might logically be expected under the conditions. Realizing that the 30 minute extraction with saturated ammonium oxalate does get materials, other than potash, that are often weighed up with the potassium chloroplatinate and that give high potash values unless corrected for, the Associate Referee will recommend that the present official method be modified by a provision to dissolve out the potassium chloroplatinate and to reweigh.

#### 1 MM. DEGREE FINENESS VERSUS $\frac{1}{2}$ MM. DEGREE FINENESS

To determine the effect of the fineness of grinding on the uniformity of potash results, four samples of fertilizer ground to represent the above degrees of fineness were sent to 13 chemists with the following instructions:

Determine the potash by the present official method, *Methods of Analysis*, A.O.A.C., 1940, 31, 42(a), on Samples MF 118, 120, 121 and 122 as follows:

A. Weigh six 2.5 gram samples from Samples 118X, 120X, 121X, and 122X and determine the potash in each of the 6 solutions of each sample.

B. Weigh six 2.5 gram samples from Samples 118Y, 120Y, 121Y, and 122Y and determine the potash in each of the 6 solutions of each sample.

*per cent*

K <sub>2</sub> O value of Samples	MF 118-12.0
	MF 120-24.0
	MF 121-20.0
	MF 122-50.0

List each individual determination of each sample by Methods A and B.

Total number of potash determinations, 48.

The uniformity of the results given in Table 2 by the nine chemists reporting indicates a decidedly better agreement with samples of  $\frac{1}{2}$  mm. fineness than of 1 mm. fineness. This agreement is better than that reported by Ford, *This Journal*, 23, 264 (1940). Table 2 also shows that closer agreements in nearly all cases favor the  $\frac{1}{2}$  mm. over the 1 mm. degree of fineness. Statistically the results show an "F" value of 1.66 for the  $\frac{1}{2}$  mm. degree of fineness, and for this comparison to be significant at 5 per cent level the "F" value should have been 1.89. The expressions of the various chemists also indicate that finer grinding for many samples is a decided benefit. The Associate Referee considers that it would not be necessary to grind many samples finer than 1 mm., which is the fineness specified by the official method. In this class might unquestionably be put such materials as superphosphate, rock phosphate, nitrate of soda, ammonium sulfate, and cyanamide. There is, likewise, a group of materials that should be ground finer than the 1 mm. now permitted. In this class fall all complete fertilizers, all mixtures of phosphate and potash, and potash salts. Most of the potash salts found on the market in Indiana have been reconditioned or at least resacked, and they also show contamination by nitrogen and phosphate. These fertilizers should be ground finer than 1 mm. if the best results are to be obtained. Therefore it will be recommended that the official method for the preparation of the sample be modified to allow for grinding finer than the 1 mm. now permitted.

#### COMMENTS OF COLLABORATORS

##### Analyst

(1) Ignitions were made in platinum dishes, in pyrometer-controlled electric muffle furnace. Final ignition temperature was 575° C. Filtrations were made through asbestos-padded Gooch crucible.

(2) For samples having a tendency to segregate would favor  $\frac{1}{2}$  mm. over the 1 mm. Grinding ordinary samples finer would prove a laborious task. Suggest that the official method specify grinding of fertilizer sample to pass through at least  $\frac{1}{2}$  mm. sieve. Sieve openings need not be circular. Better agreement of results was obtained on the Y series than on the X series.

(3) I hesitated sending results as usually better agreement is obtained on ordinary fertilizers.

(9) Ignitions were made in platinum dishes at 800°-900° C., Fisher burner. Filtrations were made through Coors Gooch crucibles on asbestos mat.

(11) Ignition was made in platinum, and final temperature of ignition is unknown. Fisher No. 3-900, high-temperature, gas burner was used. Filtrations were made through a Selas porcelain crucible with glass-sintered bottom (F.C. 30-Porosity 10).

(13) Ignitions were made in platinum dishes on Fisher-type burner with natural gas. Filtrations were made in asbestos-padded Gooch crucibles, with filter paper under asbestos pad. The official method seems to give results a little too high, or something is weighed with the precipitate that is not potash. In reporting samples of this composition I should rather take the results obtained by the washing procedure than those obtained by the official method.

(15) Ignitions were made in silica dishes at 600° C. in controlled muffle furnace.

TABLE 2.—*Effect of fineness of sample on potash results*  
1 mm. Degree of Fineness versus  $\frac{1}{2}$  mm. Degree of Fineness

ANALYST NO.	NO. OF DETER- MINATIONS	SAMPLE 118X 1 MM.			SAMPLE 118Y $\frac{1}{2}$ MM.			SAMPLE 120X 1 MM.			SAMPLE 120Y $\frac{1}{2}$ MM.		
		HIGH	LOW	AV.	HIGH	LOW	AV.	HIGH	LOW	AV.	HIGH	LOW	AV.
2	6	13.46	12.70	13.05	13.24	12.70	13.01	23.61	23.16	23.33	23.16	22.95	23.08
3	6	13.12	12.38	12.64	12.58	12.34	12.43	23.56	23.28	23.44	23.22	22.87	23.04
4	6	13.90	13.02	13.48	12.94	12.60	12.74	23.78	23.00	23.33	23.24	23.10	23.17
5	6	13.14	12.89	13.03	12.87	12.73	12.82	23.60	23.12	23.25	23.24	22.96	23.13
10	6	13.37	12.71	13.01	13.10	12.91	13.01	23.36	23.02	23.17	23.28	23.00	23.17
12	6	13.32	12.20	12.92	12.95	12.84	12.91	23.56	22.94	23.32	23.48	23.30	23.39
17	6	13.28	12.24	12.80	12.69	12.29	12.48	23.22	22.82	23.00	23.20	22.75	22.94
23	6	12.01	11.09	11.46	11.92	11.26	11.58	23.17	22.31	22.68	23.24	22.26	22.84
24	4	13.68	12.92	13.39	13.13	12.74	12.87	23.64	23.12	23.34	23.27	23.00	23.13
Av.		13.25	12.46	12.86	12.82	12.49	12.65	23.49	23.02	23.20	23.29	22.91	23.10
Stand. Dev.				.58			.45			.22			.15

		SAMPLE 121X 1 MM.			SAMPLE 121Y $\frac{1}{2}$ MM.			SAMPLE 122X 1 MM.			SAMPLE 122Y $\frac{1}{2}$ MM.		
		HIGH	LOW	AV.	HIGH	LOW	AV.	HIGH	LOW	AV.	HIGH	LOW	AV.
2	6	20.15	19.67	19.81	20.01	19.61	19.82	50.47	49.65	50.16	50.69	50.13	50.34
3	6	19.83	18.93	19.56	20.03	19.48	19.79	50.23	49.65	49.88	50.19	49.94	50.11
4	6	20.02	19.16	19.56	19.90	19.68	19.80	49.94	49.24	49.59	50.14	49.98	50.01
5	6	19.95	19.14	19.41	19.85	19.35	19.65	50.55	50.15	50.37	50.65	50.35	50.47
10	6	19.80	19.42	19.64	19.76	19.42	19.66	50.40	49.76	49.91	50.24	49.44	49.81
12	6	19.70	19.26	19.53	19.70	19.48	19.60	50.80	49.96	50.31	50.30	50.04	50.19
17	6	20.00	19.63	19.72	19.84	19.44	19.65	49.61	48.82	49.29	49.80	48.65	49.15
23	6	19.56	18.89	19.22	19.71	19.30	19.45	50.95	48.40	49.28	50.16	48.73	49.39
24	4	20.16	19.80	19.97	19.64	19.40	19.54	50.28	49.88	50.04	50.02	49.79	49.89
Av.		19.91	19.32	19.62	19.82	19.47	19.66	50.36	49.50	49.87	50.24	49.67	49.93
Stand. Dev.				.20			.13			.40			.33

Filtrations were made in an asbestos-padded Gooch, with disk of Whatman No. 5 filter paper under the asbestos. Results reported are from 2 aliquots.

(16) Ignitions were made in platinum dishes, over coffee burner and finally over Meeker. Temperature of ignition was about 600° C. Filtrations were made in Gooch crucibles with filter paper under the asbestos pad. In no case did the crucible, after the  $K_2PtCl_6$  had been washed out, weigh more than it did before the precipitate was caught on it.

(17) In the few cases where, in our regular inspection and analysis, analytical differences with manufacturers have arisen, finer grinding has not solved the difficulty. We have made no extended comparisons of this sort, however. I believe we should be slow to obligate ourselves to the extra time and labor involved in grinding samples to  $\frac{1}{4}$  mm. unless the evidence in favor of it is convincing.

(19) Ignition was made in platinum dishes on an electric furnace. Final temperature was about 800° C. Filtrations were made into asbestos-padded Gooches. In each case there was a portion that would not redissolve after ignition (probably silica). For exacting work, redissolving and reweighing are undoubtedly the more accurate.

(20) Ignition was made in porcelain crucibles. Final ignition temperature was 580° C. in an electric muffle furnace. Asbestos-padded Gooch filter was used for all filtrations.

(22) Samples were rather difficult to handle and filtered very slowly. All except MF 117 had a scale that crawled up on the sides of the evaporating dishes. None of the samples acted like the ordinary fertilizers obtained in this state.

(23) Ignition was made over Purdue burners and finally over a large Meeker burner. Filtrations were made in both asbestos-padded Gooch and Jena 1 bg4 sintered crucibles.

(24) Ignition was made in platinum over Purdue burners and finally over a Purdue Venturi Meeker burner. Final temperature was about 700°C. Filtrations were made in Jena 1 bg3 glass sinters, which were washed with hot water and reweighed to correct for any insoluble residue. Samples MF 115, 116, 117, and 119 should have produced insoluble residues in all cases. Under good ignition conditions Samples MF 118, 120, 121, and 122 should not have produced any insoluble residue.

#### COLLABORATORS

- (1) Batton, H. C., Swift & Co., Baltimore, Md.
- (2) Butt, C. A., International Agricultural Corp., Atlanta, Ga.
- (3) Caldwell, R. D., Armour Fert. Works, Atlanta, Ga.
- (4) Caldwell, Paul, and Hoffman, A. E., Darling & Co., East St. Louis, Ill.
- (5) Charlton, R. C., and Buchanan, P. J., Am. Agr. Chem., Co., Carteret, N. J.
- (6) Farrar, B., Analytical Laboratory, Trail, B. C., Canada.
- (7) Haigh, L. D., Univ. of Missouri, Columbia, Mo.
- (8) Halvorson, H. A., Dept. of Agr., St. Paul, Minn.
- (9) Hand, W. F., and Etheredge, M. P., State College, Miss.
- (10) Hare, C. L., and Moore, O. C., Dept. of Chemistry, Auburn, Ala.
- (11) Haskins, H. D., Kuzmeski, J. W., and DeRose, R. H., Agr. Exp. Sta., Amherst, Mass.
- (12) Howes, C. Clifton, Davison Chemical Corp., Baltimore, Md.
- (13) Ingham, R. E., Royster Guano Co., Macon, Ga.
- (14) Jones, W. Catesby, Dept. of Agriculture, Richmond, Va.
- (15) Kock, R. C., Swift & Co., Hammond, Ind.
- (16) Magruder, E. W., and Lineweaver, A. N., Royster Guano Co., Norfolk, Va.
- (17) Merwin, R. T., and Bailey, E. M., Agr. Expt. Sta., New Haven, Conn.
- (18) Midgley, M. C., State College, Pullman, Wash.

- (19) Smith, Richard M., Dept. of Agriculture, Tallahassee, Fla.
- (20) Struve, O. I., Eastern States Co-op. Milling Corp., Buffalo, N. Y.
- (21) Thompson, S. K., Dept. of Agriculture, Charleston, W. Va.
- (22) Walker, L. S., Agr. Exp. Sta., Burlington, Vt.
- (23) Webb, H. J., Hollis, C. H., and Foy, J. T., Clemson Agr. College, Clemson, S. C.
- (24) Hughes, C. W., and Ford, O. W., Purdue Univ. Agr. Exp. Sta., West Lafayette, Ind.

No work was done in 1940 in connection with methods for platinum recovery. This study should be continued.

C. W. Hughes and O. W. Ford<sup>1</sup> made some comparative studies of the solubility of potassium chloroplatinate in 83 per cent alcohol and in acid-alcohol at 18° C. and at 38° C. Their results, which were summarized and presented by C. W. Hughes at the Detroit Meeting of the American Chemical Society, September 9–13, 1940, indicate an increase of solubility of potassium chloroplatinate with a rise in temperature of the alcohol and acid-alcohol. In order to eliminate the effect of the temperature it was found advisable to cool the acid-alcohol mixture as well as the alcohol before using. This work indicated that it was also advisable to cool the 6 ml. acid-alcohol mixture not only before but during the 15 minute extraction of the potassium chloroplatinate with it. These studies were made on potassium chloroplatinate precipitated from pure potassium chloride and for that reason might logically be subject to the criticism that this condition might not prevail in the actual potash determination. However, for two years the Associate Referee has considered that temperature of the alcohol does have a decided effect on the solubility of potassium chloroplatinate. Since the practice of cooling the acid-alcohol mixture and the alcohol used in the potash determination has been followed in this laboratory, the Associate Referee has found that higher and more concordant potash values have been obtained even after corrections have been made for the water-insoluble residues that occasionally show up in samples. Therefore the Associate Referee will recommend that studies be made of the solvent action of acid-alcohol and alcohols on potassium chloroplatinate at more than one temperature.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That study of the four methods (or other methods) for platinum recovery published in *This Journal*, 22, 286–287 (1939), be continued.
- (2) That collaborative work be done on a number of samples to determine the effect of temperature on the solubility of potassium chloroplatinate in acid-alcohol and alcohols in the regular potash determination.
- (3) That the words, "If not, dissolve the  $K_2PtCl_6$  in hot  $H_2O$ , reweigh, and make correction for water-insoluble residue," be included in the

<sup>1</sup> To be published.

\* For report of Subcommittee A and action of the Association, see *This Journal*, 24, 46 (1941).

parentheses in the last line of 42(a), *Methods of Analysis, A.O.A.C.*, 1940, 31, so as to have that line read: "Precipitate should be completely soluble in  $H_2O$ . If not, dissolve the  $K_2PtCl_6$  with hot  $H_2O$ , reweigh, and make correction for water-insoluble residue.", (official, first action).

(4) That the words, "For fertilizer mixtures and potash salts use a sieve having circular openings 1/50" ( $\frac{1}{2}$  mm.) in diameter," be inserted before the last sentence in line 6, par. 2, section II, p. 20, *Methods of Analysis, A.O.A.C.*, 1940 (official, first action).

#### ACKNOWLEDGMENT

The writer wishes to express his gratitude to H. R. Kraybill for his counsel in connection with this work and to C. W. Hughes for assistance in making the potash determinations.

### REPORT ON ACID- AND BASE-FORMING QUALITY OF FERTILIZERS\*

By H. R. ALLEN, *Associate Referee*, and LELAH GAULT (Kentucky  
Agricultural Experiment Station, Lexington, Ky.)

The investigation conducted this year followed the recommendation of this Association that a further study be made of water-insoluble material coarser than 20 mesh before the method is applied, *This Journal*, 22, 53 (1939). Taylor and Pierre<sup>1</sup> found that the fineness of dolomitic limestone used as a constituent of mixed fertilizers had a marked effect upon the time necessary for its decomposition. They concluded that most of the limestone should pass a 60-mesh screen and all should pass a 20-mesh screen. Dawson, Snyder, Leighty, and Reid, *This Journal*, 22, 137 (1939), and Collins and Speer, *Ibid*, 142, and 23, 373 (1940), reported similar results.

It appears in this study that wet sieving is necessary in order to remove all fine limestone mixed with, or adhering to, other constituents and also the water-soluble compounds, particularly nitrogen salts. In a preliminary study on the size of sample necessary for wet sieving, the former referee, *Ibid*, 21, 302 (1938), washed samples of different weights on a 20-mesh screen with 20 times their weight of water and recommended that not less than 100 grams be used. In the present investigation a 100 gram sample was used, but the washing technic was varied.

In the development of a chemical method for elimination of material coarser than 20 mesh, the three following approaches are possible:

A.—*In Terms of the Portion Coarser than 20 Mesh.*—The basicity of the ash of the coarser than 20-mesh portion, determined by the A.O.A.C. tentative method

\* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

<sup>1</sup> *J. Am. Soc. Agron.*, 27, 764 (1935).



and multiplied by a proper factor, would be subtracted from the basicity of the ash of the whole sample.

B.—*In Terms of the Portion Finer than 20 Mesh.*—This would require only one ash determination.

C.—*In Terms of a Carbon Dioxide Determination on the Portion Coarser than 20 Mesh.*—The calcium carbonate equivalent found, multiplied by a factor, would be subtracted from the calcium carbonate equivalent of the ash of the whole sample as determined by the tentative method.

So far, work has been conducted under A and B only. These methods were applied to 4 laboratory samples, and to 27 unground inspection samples of mixed fertilizers, guaranteed non-acid-forming, or basic in reaction, which were obtained from control officials of five states. Samples 1-6, inclusive, are from Alabama, 17-22 from North Carolina, 25-30 from South Carolina, 39-44 from Georgia, and 45-47 from Mississippi. The 4 prepared laboratory samples had the following composition:

SAMPLE NUMBER	POUNDS PER TON	
	1a	2a
Ammonium sulfate	142	244
Sodium nitrate	312	128
Tankage	354	—
Superphosphate, 20%	500	1000
Superphosphate, 47%	170	—
Muriate of potash, 50%	160	160
Dolomitic limestone	362	468

Half the dolomitic limestone passed the 20-mesh screen by dry sieving, and the remainder passed through the 10-mesh screen. The dolomitic limestone used analyzed 90 per cent calcium carbonate equivalent by the tentative method. Samples 1b and 2b had the same composition as Samples 1a and 2a, except that sand, free from carbonates, was substituted for the dolomitic limestone. All the sand passed a 20-mesh sieve. Dry sieving of the other constituents gave the following percentage of each constituent that was coarser than 20 mesh: ammonium sulfate, 0.4; sodium nitrate, 50; tankage, 56; superphosphate in Samples 1a and 1b, 31; superphosphate in Samples 2a and 2b, 19; muriate of potash, 0.6.

#### EXPERIMENTAL METHODS

*Procedure I, regular tentative method.*—The tentative method for acid- and base-forming quality was applied to 4 laboratory samples and to 27 inspection samples received from control officials. The total nitrogen and citrate-insoluble phosphoric acid were determined for each sample, and the net basicity or acidity was evaluated.

*Procedure II, portion coarser than 20 mesh.*—A 100 gram portion of each sample was transferred to a 5-inch, 20-mesh sieve and separated by dry

sieving, and the part finer than 20 mesh was weighed. The portion coarser than 20 mesh was placed in a 400 ml. beaker, 100 ml. of the water was added, and the beaker was shaken several times. After 5-10 minutes, the sample was transferred to the 5-inch, 20-mesh sieve supported by the walls of a 2 liter Pyrex beaker, and was washed with a stream of distilled water to a volume of 1000 ml. Wash water was supplied from a 3 liter flask on a shelf overhead. A source of compressed air was connected with the flask by means of a tube through the stopper, and a hand-controlled clamp on the rubber tubing outlet allowed varying degrees of pressure to be applied to the washing stream. Washing was conducted with minimum pressure for about one-half the washing time and with a medium pressure during the remainder of the washing. Titration of 1000 ml. of water delivered in this way showed the absorption of carbon dioxide to be negligible. The compressed air was not passed through the wash water. The sieve containing the washed portion coarser than 20 mesh was placed in an oven, and the material was dried overnight at 75° C. Material was weighed and basicity of the ash was determined by the tentative method. A moisture determination was made on the whole sample and on the dried portion coarser than 20 mesh. The weight of this portion, or its fractional part of the whole sample, was adjusted to the moisture content of the whole sample. The basicity of the ash of the coarser than 20-mesh portion multiplied by this fraction was subtracted from the ash basicity of the whole sample, and the net basicity or acidity was evaluated by applying the correction based on the total nitrogen and citrate-insoluble phosphoric acid content of the whole sample.

*Procedure III, portion finer than 20 mesh.*—The 1000 ml. volumes from wet sieving of 4 laboratory samples and 12 of the inspection samples were evaporated to dryness on a steam hot plate, and the dry residue was scraped out and mixed with the dry sieved portion. The basicity of the ash was determined by the tentative method, and the net basicity or acidity was determined by applying the correction based on the total nitrogen and citrate-insoluble phosphoric acid content of the whole sample.

In Table 1 are given weights of the wet and dry sieved fractions and moisture content of the original samples and of the dried part coarser than 20 mesh.

In Table 2 are shown the basicity or acidity of the ash and the net basicity or acidity by the three procedures for the samples under consideration.

#### DISCUSSION OF RESULTS

By the tentative method for acid- and base-forming quality, Procedure 1, 24 of the 27 inspection samples fulfilled the guaranty, and the maximum deficiency in the other 3 samples was 53 pounds calcium carbonate. When Procedure II was applied, only 11 samples met the guaranty, while 6

others were less than 50 pounds calcium carbonate deficient. Portions of the coarser than 20-mesh part of all the remaining samples gave a strong effervescence when treated with dilute acid. In two cases where the basicity guaranty was satisfactory there was apparently present a consider-

TABLE 1.—*Sieving and moisture results*  
(Expressed as per cent)

LABORATORY NUMBER	<20 MESH DRY SIEVED	<20 MESH WET SIEVED	>20 MESH*	TOTAL RECOVERED	MOISTURE	
					WHOLE SAMPLE	>20 MESH DRIED
1a	60.0	15.5	23.7	99.2	3.59	1.93
1b	69.9	17.4	11.5	98.8	3.76	3.12
2a	69.8	10.0	19.8	99.6	3.67	.75
2b	79.8	9.7	10.0	99.5	3.69	1.40
1	80.2		15.9		6.48	2.53
2	67.7	7.6	21.0	96.3	3.79	2.02
3	72.1		18.3		4.20	2.04
4	71.2	12.0	15.3	98.5	3.33	1.33
5	74.0		15.5		4.47	0.62
6	69.7	8.9	21.4	100.0	3.96	0.78
17	79.4	7.1	12.7	99.2	3.59	2.07
18	77.6	5.0	14.8	97.4	3.97	2.56
19	88.3		6.9		3.88	1.67
20	84.6	7.0	7.6	99.2	3.63	2.14
21	86.8	6.2	6.2	99.2	4.12	1.92
22	82.2	4.2	12.6	99.0	3.83	2.22
25	81.6	8.3	9.5	99.4	2.79	2.20
26	71.5	10.1	17.2	98.8	2.85	2.37
27	71.0	11.9	16.2	99.1	2.90	2.09
28	70.8	11.3	17.2	99.3	2.61	1.63
29	85.6		7.7		2.52	1.38
30	59.3		19.5		4.37	1.74
39	75.9		14.3		4.77	1.28
40	71.5		20.6		5.02	1.25
41	76.5		12.4		5.15	1.35
42	70.4		14.5		6.82	2.03
43	86.4		9.9		4.87	1.25
44	79.5		11.0		8.26	1.27
45	75.1		14.6		5.31	2.47
46	77.9		14.2		8.27	2.81
47	75.3		15.4		8.14	2.75

\* Adjusted to moisture content of whole sample.

able amount of limestone coarser than 20 mesh but also an excess of finer limestone.

Water-insoluble organic nitrogen and citrate-insoluble phosphoric acid may be increased in the portion coarser than 20 mesh due to higher concentration of water-insoluble materials. It would be desirable to determine

how much such materials affect the basicity of this portion. Laboratory Sample 1b, in which sand was substituted for dolomitic limestone, contained about one-fourth of the nitrogen as tankage, and the nitrogen con-

TABLE 2.—*Basicity or acidity results*  
(Pounds calcium carbonate per ton)\*

LABORATORY NUMBER	GUARANTY	ASH			NET BASICITY OR ACIDITY (A)		
		TENTATIVE METHOD	PROCEDURE II†	PROCEDURE III	TENTATIVE METHOD	PROCEDURE II	PROCEDURE III
1a		419	153	376	217	64	174
1b		121	9	116	76A	85A	81A
2a		339	148	187	201	53	49
2b		58A	7	83A	191A	198A	216A
1	N‡	308	56		91	35	
2	N	278	118	195	52	66A	31A
3	N	268	107		45	62A	
4	N	187	121	133	38A	159A	92A
5	N	169	91		48A	139A	
6	N	237	140	143	14	126A	80A
17	100B	234	27	228	94	67	88
18	200B	290	30	298	147	107	155
19	N	268	35		80	45	
20	200B	455	42	380	231	189	156
21	300B	460	18	473	314	296	327
22	100B	243	21	285	117	96	159
25	400B	565	29	580	434	405	449
26	100B	260	34	243	106	72	86
27	100B	263	45	228	113	68	78
28	N	210	93	108	18	75A	84A
29	N	343	15		83	68	
30	N	177	39		17	22A	
39	N	245	119		115	4A	
40	N	81	12		13A	25A	
41	N	122	69		5A	74A	
42	N	164	23		16	7A	
43	N	215	13		61	48	
44	N	126	19		2	17A	
45	N	157	91		0	91A	
46	N	159	68		7	61A	
47	N	127	59		6	53A	

\* Acidic results are followed by A. Other results are basic.

† Ash basicity of portion coarser than 20 mesh times  $\frac{\text{weight of } >20 \text{ mesh portion}}{100}$ .

‡ Non acid-forming.

tent of the coarser than 20-mesh portion was 5.68 per cent. However, the amount deducted due to basicity of the portion coarser than 20 mesh was only 9 pounds calcium carbonate. It seems that Procedure II was satisfactory in this case.

Citrate-insoluble phosphoric acid determinations were made on the whole portions of the 27 inspection samples and on the coarser than 20-mesh portions. The average was 0.39 per cent for the whole and 1.49 per cent for the coarser than 20-mesh portions. The maximum ash basicity by Procedure II, due to increase in citrate-insoluble phosphoric acid, was 11 pounds calcium carbonate and the average increase was 4 pounds.

Results from Procedure III checked with Procedure II in only 4 of the 12 determinations made. It is believed that Procedure III is not so reliable an index of the amount of limestone coarser than 20 mesh as is Procedure II because of varying concentration of the sample through elimination of material coarser than 20 mesh. No correction of this by use of a factor is apparent. The possible inaccuracy of Procedure III is illustrated in results from laboratory Sample 1a, where an ash basicity of 376 pounds calcium carbonate and a net basicity of 174 pounds are found. The 64 pounds net basicity found by Procedure II is near the figure expected. Note that Procedures II and III checked closely for laboratory Sample 2a.

It was thought advisable to make moisture determinations on the whole samples and on the portions coarser than 20 mesh in order to place them on the same moisture basis. Results show that the maximum net basicity difference due to the different moisture content was only 5 pounds calcium carbonate and the average difference would probably be between 2 and 3 pounds. It appears that the moisture determination might be omitted. In view of the results found, it is thought that estimation of calcium carbonate equivalent in the portion coarser than 20 mesh should be studied by determination of carbon dioxide in this portion.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That study of elimination of water-insoluble material coarser than 20 mesh be continued.
- (2) That the basicity of phosphate rock and other factors that affect the method be studied further.

### REPORT ON CALCIUM AND SULFUR

By GORDON HART (Department of Agriculture,  
Tallahassee, Fla.), *Associate Referee*

#### CALCIUM

##### *Acid-Soluble Calcium*

Four methods were tested.

*Method 1* is the titration of calcium oxalate precipitate from the Bartlett-Tobey method for the determination of calcium and magnesium, *This*

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 46 (1941).

*Journal*, 22, 270 (1939). The calcium is precipitated as outlined by the Referee on Magnesia, J. B. Smith.

*Method 2* is a double precipitation as outlined in Method 1.

*Method 3* is a modification of the stock feed method, which is now tentative for the determination of calcium in fertilizers, *Methods of Analysis*, A.O.A.C., 1940, 34, 47. It eliminates the ashing of sample and makes the preparation of solution conform to that of the Bartlett-Tobey method.

*Method 4* is Method 5 of last year's collaborative work and is taken from Research paper RP 1095, National Bureau of Standards, by Hoffman and Lundell, pp. 616-617.

Two samples were prepared and sent out to collaborators, Sample 2 and Sample 3. Sample 2 contained 10.00 per cent calcium and 5.00 per cent manganese as MnO. It also contained iron, alumina, magnesia, nitrogen, phosphoric acid, and potash. Sample 3 was a mixed fertilizer.

#### METHODS USED

##### *Method 1 (Modified Bartlett-Tobey)*

Weigh 2.5 grams of fertilizer into a 250 ml. volumetric flask, add 30 ml. of HNO<sub>3</sub> and 10 ml. of HCl, and boil for 30 minutes. Cool, make to volume, and mix. Transfer an aliquot of the clear solution containing not more than 20 mg. of MgO to a 400 ml. beaker. Partially neutralize with NH<sub>4</sub>OH. Add a few drops of methyl red. Add NH<sub>4</sub>OH until the solution is yellow, then HCl until barely pink. Add 10 ml. of saturated solution of NH<sub>4</sub> oxalate for each 50 ml. of solution, adjust reaction of the solution to pH 5.0 (a faint pink color) by addition of HCl (1+4), or NH<sub>4</sub>OH (1+4), boil for a few minutes, cool, and again adjust reaction to pH 5.0, adding more methyl red if necessary. Stir thoroughly and allow the solution to stand until the precipitate settles. Filter through 11 cm. filter paper fine enough to retain Ca oxalate and wash 10 times with hot water. (Save filtrate for magnesium determination.) Place beaker in which precipitation was made under funnel, punch a hole in filter paper and wash precipitate back into beaker with small portions of dilute H<sub>2</sub>SO<sub>4</sub> (125 ml. H<sub>2</sub>O + 5 ml. H<sub>2</sub>SO<sub>4</sub>). Add all 130 ml. of dilute acid. Heat to 70° or above, add a few drops of MnSO<sub>4</sub> solution, and titrate with 0.1 N KMnO<sub>4</sub> until first pink color is obtained. Correct for blank and calculate to Ca.

##### *Method 2*

Repeat the precipitation specified in Method 1. Transfer Ca oxalate and filter paper to the beaker in which precipitation was made. Add 20 ml. of HCl (1+1), heat to boiling, stir the paper to a pulp, dilute to 50 ml. with hot water, and keep hot 20 minutes, stirring occasionally. Filter into a 250 ml. beaker and wash well with 50 ml. of hot HCl (5+95), using small portions. Add 10 ml. saturated NH<sub>4</sub> oxalate, heat to 80°-90° C., add 3 drops of bromophenol blue, and add NH<sub>4</sub>OH until the indicator changes to green but not blue. Allow to cool gradually to room temperature. Filter through a paper of fine texture, wash out beaker onto paper with hot water, and wash paper and precipitate 9 more times with small portions of hot water. Place beaker in which precipitation was made under funnel. Punch a hole in filter paper and wash the Ca oxalate into beaker with 130 ml. (125 ml. H<sub>2</sub>O + 5 ml. H<sub>2</sub>SO<sub>4</sub>), heat to about 90°, add a few drops of saturated MnSO<sub>4</sub> solution, and titrate with standard KMnO<sub>4</sub>. Calculate to Ca.

*Method 3*

Transfer a 25 ml. aliquot of the dissolved sample, prepared as directed in Method 1, to a beaker, and dilute to 100 ml. Add 2 drops of bromophenol blue indicator. Add  $\text{NH}_4\text{OH}$  (1+4) to the point where the indicator changes from yellow to green (not blue). If overrun, bring back with  $\text{HCl}$  (1+4). (This gives a pH of 3.5-4.0.) Dilute to 150 ml. Bring to boiling and add slowly with constant stirring 30 ml. of saturated hot  $\text{NH}_4$  oxalate solution. If the color changes from green to blue or yellow, again adjust to green with  $\text{HCl}$  (1+4) or  $\text{NH}_4\text{OH}$  (1+4). Digest on steam bath 1 hour, or let stand overnight, and cool to room temperature. Filter the supernatant liquid through a quantitative filter paper on a Gooch crucible or on a fritted glass filter (Jena 1G4 is preferred), and wash the precipitate thoroughly with  $\text{NH}_4\text{OH}$  (1+50). Place the filter paper or crucible with the precipitate in the original beaker and add a mixture of 125 ml. of water plus 5 ml. of  $\text{H}_2\text{SO}_4$ . Heat to  $70^\circ\text{C}$ . or above and titrate with 0.1 N  $\text{KMnO}_4$  until first slight pink color is obtained. Correct for blank and calculate to Ca.

*Method 4*

## REAGENTS

(a) *Saturated ammonium oxalate solution.*—Approximately 42 grams of  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  per liter.

(b) *Bromophenol blue indicator.*—Dissolve 0.10 gram of bromophenol blue reagent in 1.5 ml. of 0.1 N  $\text{NaOH}$  and dilute to 25 ml.

(c) *Ammonium oxalate-oxalic acid wash solution.*—Dissolve 2 grams of  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$  and 1 gram of  $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$  in water and dilute to 1 liter.

## DETERMINATION

Weigh 2.5 grams of sample into a 250 ml. volumetric flask. Add 30 ml. of  $\text{HNO}_3$  and 10 ml. of  $\text{HCl}$ , and boil 30 min. Cool, make to volume, mix, filter through a dry paper, and transfer 100 ml. to a 250 ml. beaker. Add 10 ml. of 20% citric acid solution and 35 ml. of the saturated  $\text{NH}_4$  oxalate solution. Heat to  $80^\circ$ - $90^\circ\text{C}$ ., add 3 drops of the bromophenol blue indicator and then  $\text{NH}_4\text{OH}$  until the indicator just partially changes color (pH 3.5). When the precipitate settles the solution should be green and not a distinct blue. Digest on the steam bath 1 hour. Cool to room temperature. Filter through a paper of close texture and wash the precipitate and paper 3 times with  $\text{NH}_4$  oxalate-oxalic acid wash solution. Transfer the paper and precipitate to the beaker in which the precipitation was made. Add 20 ml. of  $\text{HCl}$  (1+1), heat to boiling, stir the paper to a pulp, dilute to about 50 ml. with hot water, and digest on the steam bath about 20 minutes, stirring occasionally. Filter into a 250 ml. beaker and wash with 50 ml. of hot  $\text{HCl}$  (5+95) in small portions. Add 15 ml. of the saturated  $\text{NH}_4$  oxalate solution and 10 ml. of 20% citric acid solution and precipitate the Ca as before. Filter through an ashless paper of close texture and wash 3 times with the  $\text{NH}_4$  oxalate-oxalic acid wash solution. Transfer the paper and precipitate to a weighed Pt crucible with cover and heat in the uncovered crucible until carbon is destroyed. Cover crucible, ignite at about  $1200^\circ\text{C}$ ., and weigh as  $\text{CaO} + \text{Mn}_2\text{O}_3$ . Repeat ignition and weighing until a constant weight is obtained.

Transfer the ignited residue to a 200 ml. volumetric flask. Add 50 ml. of  $\text{HNO}_3$  (1+4), 5 ml. of 85%  $\text{H}_3\text{PO}_4$ , and 0.3 grams of  $\text{KIO}_4$ . Heat just below the boiling point for 30 minutes. Cool to room temperature and dilute to volume. Compare colorimetrically with a permanganate standard. Calculate to  $\text{Mn}_2\text{O}_3$  and subtract from weight of ignited residue.

Seven collaborators reported. Collaborators 1 and 7 reported a number

of determinations and an average. The averages are then averaged with other collaborative results to give final average. Five to seven solutions were prepared from Samples 2 and 3, denoted by the letters a, b, c to g. From these solutions aliquots were taken for all three or four methods reported on.

TABLE 1.—*Collaborative results on calcium (as Ca)*

COLLAB-ORATOR	SAMPLE 2				SAMPLE 3					
	METHOD 1	METHOD 2	METHOD 3	METHOD 4	METHOD 1	METHOD 2	METHOD 3	METHOD 4		
1	a	10.00	9.76	9.92	—	a	12.72	12.72	12.88	—
	b	10.24	10.00	10.08	—	b	12.48	12.56	12.72	—
	c	10.08	9.76	9.92	—	c	12.48	12.40	12.64	—
	d	10.16	9.84	10.00	—	d	12.72	12.64	12.80	—
	e	10.24	10.00	10.08	—	e	12.32	12.24	12.40	—
	Av.	10.14	9.87	10.00	—	Av.	12.54	12.51	12.69	—
2	9.96	9.55	10.32	9.62	12.53	12.52	12.96	12.76		
3	10.15	10.33	10.20	9.91	12.94	12.55	12.94	12.97		
4	10.05	9.95	10.03	9.95	12.72	12.76	12.65	12.77		
5	10.83	10.32	10.44	10.27	12.69	12.68	12.47	12.84		
6	10.70	10.35	10.42	10.04	12.58	12.34	12.41	12.83		
7	a	9.66	10.04	10.07	9.84	a	12.87	—	12.78	12.99
	b	9.83	10.36	10.12	—	b	12.61	—	12.70	12.96
	c	9.69	9.65	9.77	9.56	c	12.82	—	—	—
	d	9.77	9.69	9.73	9.51	d	12.70	—	—	—
	e	10.07	10.25	10.21	10.04	e	12.59	13.07	12.99	12.90
	Av.	9.81	9.998	9.98	9.74	f	12.66	12.99	12.83	12.91
General Average	10.23 10.05 10.19 9.92				g	12.43	12.96	12.96	12.93	
					Av.	12.67	13.01	12.85	12.94	
					12.67	12.62	12.71	12.85		

Sample 2, with known calcium content, although carefully prepared, did not give uniform results. However, the results from the same solutions, a to g, indicate that the methods checked fairly well.

The average results from Method 1 and Method 3 are close, and there is not enough difference between results from Methods 1 and 3 and from Method 2 to show the need of a double precipitation if the calcium oxalate is to be titrated.

Several of the collaborators reported trouble in removing all the precip-



itate from the filter paper in Methods 1 and 2. Three collaborators preferred Method 3, one preferred Method 1.

The collaborators are W. Y. Gary, E. B. Loyless, W. Catesby Jones, L. W. Purdy, E. T. Hord, G. L. Smith, and E. J. Deszyck. Appreciation is extended for their cooperation.

### CONCLUSIONS

- (1) Methods 1 and 3 can be used satisfactorily.
- (2) Manganese does not interfere sufficiently to affect results when calcium oxalate is titrated with potassium permanganate, provided the sample has been oxidized with nitric and hydrochloric acids when the solution is prepared.
- (3) Although Method 3 is preferred, Method 1 can be used satisfactorily when magnesium is to be determined.
- (4) The filter paper with the calcium oxalate on it should be placed in a beaker for titration instead of an attempt being made to wash the calcium oxalate from the paper.

### RECOMMENDATIONS\*

It is recommended—

- (1) That Methods 1, 3, and 4 be further studied collaboratively.
- (2) That Method 1 be adopted as tentative and corrected to read as follows:

Place the Ca oxalate and filter paper from acid-soluble magnesium, p. 36, 53 ("filter through 11 cm. paper fine enough to retain Ca oxalate . . .") in beaker in which precipitation was made and add a mixture of 125 ml. of water plus 5 ml. of  $\text{H}_2\text{SO}_4$ . Heat to  $70^\circ$  or above and titrate with 0.1 *N*  $\text{KMnO}_4$  until first slight pink color appears. Correct for blank and calculate to Ca.

- (3) That the tentative acid-soluble method for calcium, *Methods of Analysis, A.O.A.C.*, 1940, page 34, 47, be changed to read as follows and remain tentative:

Weigh 2.5 grams of sample into 250 ml. volumetric flask, add 30 ml. of  $\text{HNO}_3$  and 10 ml. of  $\text{HCl}$ , and boil 30 min. Cool, make to volume, and mix. Filter if necessary. Transfer to a beaker a 25 ml. aliquot of dissolved sample, and dilute to 100 ml. Add 2 drops of bromophenol blue indicator. Add  $\text{NH}_4\text{OH}$  (1+4) to the point where the indicator changes from yellow to green (not blue). If overrun, bring back with  $\text{HCl}$  (1+4). (This gives pH of 3.5–4.0.) Dilute to 150 ml. Bring to boiling, and add slowly with constant stirring 30 ml. of saturated hot  $\text{NH}_4$  oxalate solution. If the color changes from green to blue or yellow again, adjust to green with the  $\text{HCl}$  or the  $\text{NH}_4\text{OH}$ . Digest on steam bath 1 hour or let stand overnight, and cool to room temp. Filter the supernatant liquid through a quantitative filter paper on Gooch crucible or on fritted glass filter (Jena 1G4 preferred), and wash precipitate thoroughly with  $\text{NH}_4\text{OH}$  (1+50). Place filter paper or crucible with precipitate in

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 46 (1941).

original beaker and add mixture of 125 ml. water plus 5 ml. of  $\text{H}_2\text{SO}_4$ . Heat to  $70^\circ$  or above and titrate with 0.1  $N$   $\text{KMnO}_4$  until first slight pink color is obtained. Correct for blank and calculate to Ca.

### SULFUR

Three samples were sent out to collaborators. Two methods for the determination of total sulfur and one for sulfate sulfur were outlined for study.

Three collaborators reported results. The Referee regrets to acknowledge that he did not get time to run the samples himself.

The total sulfur found by either Method 1 or Method 2 less the sulfur found by Method 3 should have equaled free sulfur.

The methods follow:

#### TOTAL SULFUR

##### *Method 1*

Place 1 gram of sample in a 250 ml. beaker, add 10 ml. of a saturated solution of Br in  $\text{CCl}_4$ , cover, and allow to stand about 30 minutes, stirring several times. Add 15 ml. of  $\text{HNO}_3$ , cover, and allow to stand about 30 minutes, stirring several times. Evaporate on the hot plate to about 5 ml. Add 20 ml. of  $\text{HCl}$  and evaporate to about 5 ml. Add about 50 ml. of water, filter, and wash with 2%  $\text{HCl}$ . Add 2 drops of 0.4% bromophenol blue solution and then  $\text{NH}_4\text{OH}$  to the first color change of the indicator. Add 10 ml. of 20% citric acid solution, dilute to 150 ml., heat to boiling, and add 10%  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  solution slowly dropwise until about 50% excess is present (1 ml. of 10%  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  solution will precipitate about 0.013 gram of S). Cover, and digest on the steam bath for at least 1 hour. Remove from steam bath and allow to cool to room temperature. Filter through asbestos into a Gooch that has been ignited at  $500^\circ \text{C}$ . and weighed, or on quantitative filter paper (Munktell No. 00). Wash 10 times with hot water. Ignite in a muffle at  $500^\circ \text{C}$ . for at least 30 minutes. Cool in a desiccator and weigh as  $\text{BaSO}_4$ . Calculate and report as S.

##### *Method 2*

Weigh 1 gram of sample and transfer to 400 ml. Pyrex beaker. Place the beaker in a bath of cold water, add 30 ml. of fuming  $\text{HNO}_3$ , cover with watch-glass, and allow to stand 15 minutes. Transfer beaker to steam bath and heat 2 hours or until S is completely oxidized. Remove watch-glass and evaporate to dryness, add a few ml. of  $\text{HCl}$ , and again evaporate to dryness to drive off nitrates. Take up with 100 ml. of water and 3-4 ml. of  $\text{HCl}$ . Filter, and wash to 200 ml. into a 400 ml. beaker. Add 2 or 3 drops of bromophenol blue and neutralize with  $\text{NH}_4\text{OH}$ . Make acid with  $\text{HCl}$ , adding a drop at a time. Add 1 ml. of  $\text{HCl}$ , heat to gentle boil, and add sufficient 10%  $\text{BaCl}_2$  dropwise to precipitate the S. (If boiling gently when the  $\text{BaCl}_2$  is added it will not be necessary to stir.) Boil gently 15 minutes, then set on steam bath 1 hour. Filter, and wash 10 times with hot water. Ignite, and weigh as  $\text{BaSO}_4$ . Calculate and report as S.

#### SULFATE SULFUR

##### *Method 3*

Weigh 2.5 grams of sample into a 250 ml. flask, add 30 ml. of  $\text{HCl}$  (1+1), boil 15 minutes, add 150 ml. of water, and boil 5 minutes. Cool, make up to mark, shake thoroughly, and filter through dry filter. Take a 50 ml. aliquot in 400 ml.

beaker, dilute to about 200 ml., neutralize with  $\text{NH}_3$ , using bromophenol blue indicator, and add 1 ml. of  $\text{HCl}$ . Heat to gentle boiling and precipitate with 10%  $\text{BaCl}_2$  dropwise. Continue gentle boiling for 15 minutes, then allow to stand on water bath 1 hour. Filter, and wash 10 times with hot water. Ignite, and weigh as  $\text{BaSO}_4$ . Calculate and report as S.

TABLE 2.—*Collaborative results on sulfur**Sample 1*

ANALYST NO.	METHOD 1	METHOD 2	METHOD 3 SULFATE SULFUR	A FREE SULFUR	B FREE SULFUR
1	12.23	12.19	7.28	4.95	4.91
2	12.02	12.04	7.32	4.70	4.72
3	12.78	—	7.78	5.00	—
Average	12.34	12.11	7.46	4.88	4.81

*Sample 2*

1	7.05	7.12	6.22	—	—
2	7.62	7.60	7.32	—	—
3	8.02	—	7.86	—	—
Average	7.58	7.36	7.13	—	—

*Sample 3*

1	6.01	5.84	5.58	—	—
2	5.46	5.37	5.39	—	—
3	5.97	—	5.73	—	—
Average	5.81	5.60	5.56	—	—

Allan J. Weaver, L. W. Purdy, E. L. Hord, and G. L. Smith did the collaborative work. Appreciation is hereby expressed to them.

The results do not indicate that either method is satisfactory. The free sulfur found by difference is low. Sample 1 contained 12.10 per cent total sulfur, 5 per cent of which was free sulfur; Sample 2 contained 7.10 per cent total sulfur; and Sample 3 had an unknown content of sulfur.

It is recommended that methods for the determination of sulfur in mixed fertilizers be studied.

## REPORT ON COPPER AND ZINC

By W. Y. GARY (Department of Agriculture,  
Tallahassee, Fla.), *Associate Referee*

The range of the quantities of copper and zinc commonly found in fertilizers covers the upper limits at which analyses for traces of these elements are generally accurate and the lower limits of accuracy for the standard methods for large amounts of the elements concerned. In the collaborative work this year consideration was given to the range within which the methods being tried may be considered accurate. Samples were therefore prepared to cover the borderline between the two types of analyses.

As a base material for preparing the samples a 4-8-4 commercial fertilizer mixture made from nitrate of soda, sulfate of ammonia, ammonium phosphate, fish meal, Peruvian guano, superphosphate, and muriate of potash was used. This mixture was not supposed to contain any added copper or zinc. However, all mixed fertilizers are likely to contain traces of these elements. Lundstrom and Mehring (1) analyzed 44 samples of mixed fertilizers chosen to be representative of the various classes and grades of commercial fertilizers and collected from manufacturers and state control officials in 16 states in 1935. They found 0.001-0.017 per cent cupric oxide with an average of 0.006 per cent and 0.000-0.075 per cent zinc oxide, with an average of 0.022 per cent. Consequently the fertilizer to which copper and zinc were to be added for the collaborative samples was analyzed by several methods sensitive to small quantities of these elements. The results are shown in Table 1.

TABLE 1.—*Results of analyses of the fertilizer used as a base in preparing samples for the Cu and Zn collaborative work*

METHOD	Cu	Zn
	<i>per cent</i>	<i>per cent</i>
Spectrographic	0.015	0.029
Dithizone	—	.010
Diethyldithiocarbamate	.005	—
Iodometric	.004	—
Gravimetric	—	.008
Average	.008	.016

The analyses indicated in Table 1 were made as follows:

*Spectrographic Method by L. H. Rogers, College of Agriculture, University of Florida* (2).—Rogers also made spectrographic analyses of Sample 1, which was sent to the collaborators. He reported 0.10 per cent of copper and 0.13 per cent of zinc, which compares well with the theoretical percentages, 0.115 and 0.129 per cent, respectively, which were calculated

from the quantities found spectrographically in the base material plus the quantities of added copper and zinc.

*Dithizone method.*—Analyses for zinc were made by Hale Cowling, Associate Referee on Zinc in Plants, who used the method of Miller and Cowling, which was also submitted to the collaborators on zinc in plants this year.

*Diethyldithiocarbamate method.*—Analyses for copper were made by W. Y. Gary, who used a method furnished by Lillian I. Butler, Associate Referee on Copper in Plants.

*Iodometric method.*—Analyses for copper were made by E. B. Loyless, Jr., Florida Department of Agriculture, who used the volumetric method essentially as sent to the collaborators except that a 10 gram sample was taken.

*Gravimetric method.*—Analyses for zinc were made by E. B. Loyless, Jr., who used a 10 gram sample, precipitated the copper and zinc sulfides together between pH 2.0 and pH 3.0, determined the combined oxides by ignition at 950° C., then subtracted the weight of cupric oxide calculated from the volumetric copper determination and assumed the remainder to be zinc oxide.

To portions of the base material were added the following C.P. salts:  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ , and  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , in sufficient amounts so that the percentages of added Cu, Zn, Mn, and Fe were in the same proportions and amounted to 0.10 per cent in Sample 1, 0.20 per cent in Sample 2, 0.50 per cent in Sample 3, and 2.00 per cent in Sample 4. The averages shown in Table 1 were used in calculating the theoretical compositions of the samples. The separate results shown in Table 1 are not in close agreement, but the variations from the averages do not seem to be significant in the analysis of samples containing these quantities of copper and zinc.

Portions of the 4 samples so prepared were sent to the collaborators, with the following methods.

#### TOTAL COPPER

##### Volumetric Method

#### REAGENTS

(a) *Standard sodium thiosulfate solution.*—Dissolve 7.82 grams of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  and dilute to 1 liter.

(b) *Standard copper nitrate solution.*—Place 2.000 grams of pure Cu (electrolytic) in 1 liter volumetric flask, add 100 ml. of  $\text{HNO}_3$ , heat until the Cu is dissolved, and dilute with water to volume at room temperature.

(c) *Potassium iodide solution.*—Dissolve 50 grams of KI in enough water to make 100 ml. of solution.

(d) *Starch solution.*—Mix about 1 gram of soluble starch with enough cold water to make a thin paste; add 100 ml. of boiling water, and boil while stirring for about 1 minute.

## DETERMINATION

Weigh 2 grams of sample if less than 5% Cu; if more than 5% Cu, weigh a sufficient quantity to furnish a little less than 0.1000 gram of Cu. Place sample in a 300 ml. Erlenmeyer flask, and add 5–10 ml. of  $\text{HNO}_3$  and 7.0 ml. of  $\text{H}_2\text{SO}_4$ . Digest on hot plate to dense white fumes. If the solution becomes dark due to organic matter, cool somewhat, add a little more  $\text{HNO}_3$ , and digest again to dense white fumes, repeating the operation if necessary until the organic matter appears to be destroyed. Cool, and add 25–30 ml. of water. Boil 1 minute, remove from hot plate, and stir occasionally for approximately 15 minutes. Filter into 250 ml. Erlenmeyer flask and wash filter and residue 6 times with small portions of hot water. Cool to room temperature and dilute to 100 ml.

Pass  $\text{H}_2\text{S}$  through the solution for 10–15 minutes. Prepare a wash solution of 10 ml. of  $\text{H}_2\text{SO}_4$ , plus enough water to make 1 liter, and saturate with hydrogen sulfide. Filter the sample solution through a paper of fine texture and wash paper and precipitate 7 times with small portions of the wash solution, keeping filter funnel covered with a watch-glass as much of the time as possible. Reserve the filtrate for the Zn determination.

Place paper and precipitate in a glazed porcelain crucible and ignite at dull red heat until the C is completely destroyed. Blow the  $\text{H}_2\text{S}$  gas out of the precipitation flask and wash the  $\text{CuS}$  from the  $\text{H}_2\text{S}$  delivery tube into flask with bromine water. Add 5 ml. of  $\text{HNO}_3$  to the  $\text{CuO}$  in the cold crucible and warm until the  $\text{CuO}$  is dissolved. (This may require 10 minutes, at end of which time insoluble specks may be disregarded.) Wash the solution into precipitation flask with water and dilute to 35 ml. For standardizing the  $\text{Na}_2\text{S}_2\text{O}_3$  solution, add to another 250 ml. Erlenmeyer flask an aliquot of the standard  $\text{Cu}(\text{NO}_3)_2$  solution and more  $\text{HNO}_3$ , so that an equivalent of 5 ml. of  $\text{HNO}_3$  shall be present, and dilute to 35 ml. Hereafter treat all solutions alike. Add an excess of bromine water and a few glass beads. Boil until excess bromine is entirely expelled and the volume is less than 30 ml. Cool a little, and add  $\text{NH}_4\text{OH}$  cautiously until mixtures is distinctly alkaline. Boil until the odor of  $\text{NH}_3$  is very faint. Add 5 ml. of glacial acetic acid and boil a minute longer. Cool to room temperature and dilute to 25–30 ml. Add 2 ml. of the KI solution and titrate with the  $\text{Na}_2\text{S}_2\text{O}_3$  solution to a light yellow color. Add about 1 ml. of the cold starch solution and continue the titration to disappearance of starch-iodine color. Report as % Cu.

*Colorimetric Method*

## REAGENT

*Standard copper sulfate solution.*—Dissolve 3.929 grams of pure  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in water and dilute to 500 ml. 1 ml. = 2 mg. of Cu.

## DETERMINATION

Proceed as directed in the volumetric determination through the filtration and washing of the  $\text{CuS}$ . Place the filter paper and  $\text{CuS}$  in the flask in which the precipitation was made, add 3 ml. of  $\text{H}_2\text{SO}_4$ , and mix until the paper is thoroughly disintegrated, warming if necessary. Wash the  $\text{CuS}$  off the  $\text{H}_2\text{S}$  delivery tube into the flask with a little bromine water. Add 5–10 ml. of  $\text{HNO}_3$  and digest on hot plate to dense white fumes. Cool somewhat, add a little more  $\text{HNO}_3$ , digest again to dense white fumes, and repeat procedure until the organic matter appears to be destroyed. Wash down the sides of the flask with a little  $\text{H}_2\text{O}$ , add 1–2 ml. of 30%  $\text{H}_2\text{O}_2$ , and evaporate to dense white fumes. Cool somewhat, add 15–20 ml. of water, cool to room temperature, and add  $\text{NH}_4\text{OH}$ , approximately 1 ml. at a time, cooling under the water tap between additions, until the solution is distinctly alkaline. Add 10 ml. of  $\text{NH}_4\text{OH}$ , wash into a 50 ml. volumetric flask, dilute to mark, and mix well.

Place aliquots of the standard  $\text{CuSO}_4$  solution in 250 ml. Erlenmeyer flasks (suggested: 2.5, 5, 10, 20, and 40 mg. Cu). Add 2 ml. of  $\text{H}_2\text{SO}_4$  and dilute to approximately 20 ml. Add  $\text{NH}_4\text{OH}$ , approximately 1 ml. at a time, cooling under the water tap between additions, until the solution is distinctly alkaline. Add 10 ml. of  $\text{NH}_4\text{OH}$ , wash into a 50 ml. volumetric flask, dilute to mark, and mix well. Set the photoelectric colorimeter at the zero reading, using a cell of clear water. Determine the readings of the standards and plot on graph paper. Determine the readings of the sample solutions, using the same zero cell and compute the quantity of Cu from the standard graph.

If a photoelectric colorimeter is not available, but another type is available, prepare standards in the same manner and compare by standard procedure. If no colorimeter is available the following method may be used: Wash the ammoniacal solution into a 50 ml. Nessler tube and dilute to mark. Into a matched Nessler tube place 20 ml. of  $\text{NH}_4\text{OH}$  (1+1). Add standard  $\text{CuSO}_4$  solution from a buret or Mohr pipet, stirring well, until the color seems to be of the same intensity as that in the sample tube. Dilute to mark for final comparison. Dilute solutions are best compared by looking down into the Nessler tube held above a smooth white surface. Report as % Cu.

#### TOTAL ZINC

##### *Gravimetric Method*

Evaporate the combined filtrate and washings from the  $\text{CuS}$  precipitation (preceding method) in a 250 ml. Erlenmeyer flask to about 80 ml. Cool, and add 2 drops of 0.4% bromophenol blue solution and then  $\text{NH}_4\text{OH}$  from a dropper to first distinct color change of indicator. Cool to room temperature and add 10 ml. of 20% citric acid solution. Fit the flask with a 2-holed rubber stopper and glass tubes, one of which almost touches the bottom of the flask and the other just extends through stopper. Pass a rapid stream of  $\text{H}_2\text{S}$  through the solution for 45 minutes. Prepare a solution containing 0.5 gram of citric acid per liter and saturate with  $\text{H}_2\text{S}$ . Filter the sample solution through ashless paper of fine texture. Use a rubber policeman to loosen precipitate sticking to flask and delivery tube and wash onto filter with a jet of the prepared wash solution. Wash the paper and precipitate 7 more times with small quantities of the wash solution, keeping the funnel covered with a watch-glass as much of the time as possible. Place the paper and precipitate in a Pt crucible that has been ignited and weighed with cover. Ignite in the uncovered crucible at a low temperature, preferably in a muffle, until the paper is oxidized, then at  $900^\circ\text{--}950^\circ\text{C}$ . for 1 hour. Place cover on crucible while hot, cool in desiccator, and weigh as  $\text{ZnO}$ . Calculate to Zn.

##### *Volumetric Method*

#### REAGENTS

- (a) *Potassium ferricyanide solution.*—Dissolve 1 gram of  $\text{K}_3\text{Fe}(\text{CN})_6$  in 100 ml. of water.
- (b) *Diphenylamine solution.*—Dissolve 1 gram of diphenylamine in 100 ml. of  $\text{H}_2\text{SO}_4$ .
- (c) *Standard potassium ferrocyanide solution.*—Dissolve 8 grams of  $\text{K}_4\text{Fe}(\text{CN})_6$  in 1 liter of water.
- (d) *Standard zinc chloride solution.*—Dissolve 2.000 grams of C.P. Zn in an excess of  $\text{HCl}$  and dilute to 1 liter.

#### DETERMINATION

Precipitate  $\text{ZnS}$  as directed in the gravimetric procedure. Filter through a paper of fine texture and wash 7 times with small quantities of 2%  $\text{NH}_4\text{CNS}$ . Place the flask in which the precipitation was made under the funnel. Wash the  $\text{ZnS}$  from the

delivery tube onto the filter with HCl (1+2), and wash the filter 10 more times with small quantities of the same acid. For standardizing the  $K_4Fe(CN)_6$  solution place an aliquot of the standard  $ZnCl_2$  solution in a 250 ml. Erlenmeyer flask. To all solutions add 2 ml. of  $H_2SO_4$ , evaporate to dense white fumes, and keep fuming for about 5 minutes, but not hot enough to evaporate to dryness. Cool, add 30 ml. of water, and boil 1 minute. Cool to room temperature. Add 2 drops of the diphenylamine solution and 2 drops of the  $K_4Fe(CN)_6$  solution. Mix well and wait, if necessary, until the purple color develops. Titrate slowly with the standard  $K_4Fe(CN)_6$  solution. (At the beginning of the titration and toward the end the color will be purple, and intermediately the color will be deep blue for large quantities of Zn.) Toward end of titration titrate 1 drop at a time until the purple color disappears for at least 30 seconds. Report as % Zn.

#### COLLABORATORS

The following collaborators submitted results:

- (1) Hale Cowling, Agr. Exp. Sta., State College, East Lansing, Mich.
- (2) G. S. Fraps and T. L. Ogier, Agr. Exp. Sta., College Station, Texas.
- (3) K. T. Holley and T. G. Dulin, Agr. Exp. Sta., Experiment, Ga.
- (4) Edward J. Deszyck, Agr. Exp. Sta., Kingston, R. I.
- (5) W. H. MacIntire, L. J. Hardin, and J. W. Hammond, Agr. Exp. Sta., Knoxville, Tenn.
- (6) Seth S. Walker, Thornton and Company, Tampa, Fla.
- (7) Allen J. Weaver, Southern Analytical Laboratory, Jacksonville, Fla
- (8) E. B. Loyless, Jr., Dept. of Agriculture, Tallahassee, Fla.
- (9) C. V. Marshall and R. Payfer, Dept. of Agriculture, Ottawa, Canada.
- (10) Oscar I. Struve, Eastern States Co-op Milling Corp., Buffalo, N. Y.
- (11) Philip McG. Shuey, Shuey and Company, Savannah, Ga.
- (12) R. Vilá Mayo and Alfonso Piera, Dept. of Agriculture and Commerce, Rio Piedras, P. R.

To these collaborators the Associate Referee extends his thanks and appreciation for their generous cooperation. Appreciation is also extended to J. J. Taylor, State Chemist, for permission to carry on this work in the Florida State Chemical Laboratories.

#### VOLUMETRIC METHODS FOR COPPER

Last year, Gordon Hart, who was then Associate Referee on Copper and Zinc in Fertilizers, recommended that the copper method specifying the separation of copper with hydrogen sulfide and subsequent iodometric determination be adopted as tentative with a view to final adoption (3). This method was studied by the present Associate Referee and modified somewhat before it was submitted for collaborative work this year. Instead of diluting the digested solution in a volumetric flask and taking an aliquot, he used the entire solution and thereby shortened the method. Since the solution for low-analysis samples represents 2 grams instead of 1 gram as formerly, the accuracy for low-analysis samples is increased because more copper is available. In the preparation of the final solution to be titrated steps were adapted from Low (4) that make it unnecessary to add a measured amount of standard copper solution to the unknown sample solution



TABLE 2.—*Collaborators' results on copper and zinc (per cent)*

COLLABORATORS	SAMPLES							
	1	2	3	4	1	2	3	4
	COPPER							
	VOLUMETRIC				COLORIMETRIC			
Cowling	0.10	0.21	0.49	1.99	0.12	0.23	0.54	2.07
Deszyck	.09	.21	.50	2.01	.12	.19	.52	2.00
Dulin	.09	.20	.49	1.96	.10	.22	.48	1.65
Gary	.10	.21	.50	1.98	.13	.22	.52	2.03
Hammond-Hardin	.07	.18	.45	1.98	.07	.25	.63	2.12
Loyless	.12	.19	.50	2.03	.12	.22	.52	1.99
Ogier	.10	.21	.51	2.08	.08	.16	.48	2.22
Payfer	.11	.21	.48	2.02	.07	.22	.52	2.06
Piera	.10	.20	.49	1.99	.16	.21	.55	1.97
Shuey	—	—	—	—	.13	.22	.46	2.00
Struve	.11	.21	.50	1.98	.11	.22	.50	2.00
Walker	.07	.21	.52	1.96	.07	.14	.45	1.85
Weaver	.11	.21	.50	1.98	.07	.14	.54	1.75
Av.	.10	.20	.49	2.00	.10	.20	.52	1.98
Theoretical	.11	.21	.51	2.00	.11	.21	.51	2.00
Av. Dev. from Mean	.01	.01	.01	.03	.03	.03	.03	.11

	ZINC							
	GRAVIMETRIC				VOLUMETRIC			
Cowling	0.04	0.23	0.53	2.11	0.09	0.19	0.50	2.05
Deszyck	.13	.23	.54	2.10	.12	.20	.52	2.12
Dulin	—	.30	.58	1.97	.02	.21	.47	2.31
Gary	.11	.23	.50	2.05	.08	.19	.49	1.96
Hammond-Hardin	.16	.27	.56	2.09	.19	.23	.46	1.89
Loyless	.09	.21	.51	2.08	.10	.20	.53	2.00
Ogier	.16	.28	.58	2.17	—	—	—	—
Payfer	.12	.19	.53	2.04	.04	—	—	—
Piera	.20	.29	.60	2.06	—	—	—	—
Shuey	.10	.23	.54	2.13	—	—	—	—
Struve	.12	.23	.57	2.09	.12	.21	.50	2.07
Walker	.13	.22	.55	2.05	.12	.27	.57	1.98
Weaver	.06	.27	.55	2.14	.05	.34	.63	2.02
Av.	.12	.24	.55	2.08	.09	.23	.52	2.04
Theoretical	.12	.21	.51	2.01	.12	.21	.51	2.01
Av. Dev. from Mean	.03	.03	.02	.04	.04	.04	.04	.08

if the copper present is less than the equivalent of 0.0100 gram of cupric oxide. The addition of more copper to the solution was the most serious objection to the method used last year. No adverse comments were made on the volumetric copper method submitted this year. The results (Table 2) show good agreement among most of the analysts.

In addition to the volumetric copper determination submitted to the collaborators, the Associate Referee tried a number of modifications as follows:

(1) The filter bearing the copper sulfide, obtained as directed in the volumetric method, was washed with bromine water, until the copper sulfide was oxidized, then washed with dilute nitric acid, and all washings were drained into the flask in which the precipitation was made. The solution was then boiled down to approximately 5 ml. and diluted to 35 ml., and the method was continued as written. The procedure was tried on a number of other samples in addition to the collaborative samples and found to be satisfactory when the quantity of copper being determined was less than 10 mg. Above this amount the number of treatments with bromine water and the evaporation of the washings required more time than the ignition procedure. With over approximately 50 mg. of copper the oxidation of the copper sulfide was incomplete within any reasonable time.

(2) The filter paper and copper sulfide were digested as directed in the colorimetric procedure. After being brought to fumes of sulfuric acid the last time the solution was diluted to approximately 30 ml., and boiled a few minutes, then completed by the volumetric procedure submitted, beginning with the addition of ammonium hydroxide. This method gave a premature end point, there being further liberations of iodine each time soon after the several additional amounts of thiosulfate had been added after the temporary end points.

(3) The procedure in Scott (6), specifying the thiosulfate precipitation of copper sulfide was tried. This procedure eliminates the odor of hydrogen sulfide, but sulfur dioxide is produced instead and the solutions should be kept under a hood when being heated. The results were a little less precise than when hydrogen sulfide is used in the separation but the averages were very good.

(4) Samples were digested by the 1940 modified Bartlett-Tobey method for magnesia (see preceding report), made to volume, and filtered through dry paper; 100 ml. aliquots were neutralized to bromophenol blue with ammonium hydroxide, then 3 ml. of hydrochloric acid was added, and the procedure was continued as directed in the 1940 volumetric method for copper beginning with the hydrogen sulfide separation (see preceding report). The results were satisfactory, but there is no saving of time and the method can not be continued satisfactorily for the zinc determination.

(5) The short iodide method for copper, Scott (6), using potassium fluoride to prevent interference of iron, seemed to offer possibilities of being adapted to fertilizer analysis. This method will be investigated further.

(6) Samples were analyzed by the methods in Scott (6) and Low (4) but more nitric acid was required in the digestion to complete the oxidation of the organic matter. These methods use aluminum for separating most of the copper, then require hydrogen sulfide for separating the last trace of copper. These methods are written for copper in ores, and it has been the experience in this laboratory that for fertilizer samples, which generally contain less copper than the ores, the volumetric method submitted to the collaborators is better suited to routine work.

### COLORIMETRIC METHODS FOR COPPER

Since objections were made last year to the volumetric method when applied to low-analysis samples, it was decided to investigate colorimetric methods for small quantities of copper. Allen J. Weaver had previously used the cupric-ammonium color in estimating copper in fertilizers containing moderate quantities of copper. The method is simple and uses chemicals commonly found in laboratories.

In fertilizer analyses, it is not likely that quantities of copper less than 0.01 per cent will be significant. Since a 2 gram sample is a convenient size for digestion, this amount was selected for use, thus furnishing 0.2 mg. of copper for the lower limit of the determination. A standard containing 0.2 mg. of copper was prepared according to the procedure shown in the method submitted. This standard had a distinctly visible blue color when compared with a blank by looking down tall-form, 50 ml., matched Nessler tubes. The method should, therefore, be applicable to the lower limit of copper determinations in fertilizers.

The method follows the volumetric method through the separation of the copper sulfide. The filter paper and copper sulfide are digested with sulfuric and nitric acids and hydrogen peroxide and then the color is produced in a manner developed from a study of the procedures in Scott (7) and Snell (10).

The results obtained by the colorimetric method (Table 2) show that the averages are very close to the theoretical and to the averages obtained by the volumetric method, but that the results of the different collaborators are not in as consistent agreement as those obtained by the volumetric method. This lack of precision is a serious objection to adopting the method in its present form for general use.

The production of a greenish rather than a pure blue color was a source of error in the method. It was the experience in this laboratory that sometimes the color was greenish and sometimes blue without any apparent reason. It is indicated by Scott (7) that the quantities of reagents and the temperature of the solution before the ammonia is added influence the color produced in the ammoniacal solution.

J. J. Hardin and J. W. Hammond advocate the ignition of the filter paper and copper sulfide and solution of the residue in nitric acid as in the volumetric procedure, then continuation with the addition of ammonia. They commented as follows:

Complete destruction of organic matter by the wet digestion procedure could not be accomplished, even after several repetitions, and the final solution was greenish in color. The ignition and subsequent preparation of the solution was much easier and more rapid; spattering was avoided, and the final solution was invariably free of the green color. The results obtained are consistently higher where the wet digestion was used, indicating that the trace of color from the undecomposed organic matter resulted in excessive interference in the colorimeter.

Comparative results by Hardin and Hammond by the two methods are shown in Table 3. The results by the ignition method are nearer the theoretical than those obtained by the acid digestion method.

TABLE 3.—*Comparison of wet-ashing with dry-ashing method of treating the filter paper and copper sulfide in the colorimetric determination of copper*  
(Data furnished by J. J. Hardin and J. W. Hammond.)

SAMPLE	1	2	3	4
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Wet ash	0.07	0.25	0.63	2.12
Ignition	0.07	0.20	0.53	2.07
Theoretical	0.108	0.208	0.507	2.003

Hale Cowling commented as follows:

Is it not likely that the differences in hue are due to the presence of colored substances other than copper in the solution? These substances might be adsorbed or occluded by the CuS precipitate. If this is the case, the use of a filter which transmits a narrow band of light in the region of the spectrum where absorption by cuprammonium ions is greatest would tend to minimize the error due to the presence of these foreign colored substances. . . . Other advantages of the use of filters is a linear relation between log % transmission and concentration of copper and a greater sensitivity of the instrument to differences in copper content.

Cowling presented curves substantiating the last statement quoted. Comparisons of results obtained with and without the use of a color filter are given in Table 4. They show greater accuracy with the use of the filter.

TABLE 4.—*Comparison of results obtained with and without use of filter*  
(Cornung No. 348 red shade yellow filter)  
(Data furnished by Hale Cowling.)

SAMPLE	1	2	3	4
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Without filter	0.124	0.228	0.539	2.07
With filter	0.118	0.221	0.532	2.03
Theoretical	0.108	0.208	0.507	2.003

E. J. Deszyck commented as follows:

At the time of comparison the  $\text{NH}_4\text{OH}$  concentration is 20%, with the result that the procedure is not a very pleasant one. Can the excess  $\text{NH}_4\text{OH}$  be lowered?

The amount of ammonium hydroxide prescribed was that advised by Snell (10). Scott (7) gives methods requiring lower percentages of excess ammonia. The method submitted may be successfully modified to use less ammonia but this point should be investigated before any change is made.

#### ELECTROLYTIC METHODS FOR COPPER

Last year the Associate Referee recommended that electrolytic methods for copper be studied. The method used last year was unsatisfactory (3). The study this year was mostly of the literature. No methods were found that appeared to be any faster than the volumetric method, and as all available methods are limited to materials containing appreciable amounts of copper, they are unsatisfactory for low analysis fertilizers (5).

#### GRAVIMETRIC METHODS FOR ZINC

The Associate Referee last year recommended that Method 2 for zinc, submitted to the collaborators at that time, be further studied (3). The method was changed to fit in with the copper method as modified this year. This means primarily the use of a larger sample for low-analysis fertilizers, in order to furnish more zinc with which to work. As the method was written last year, the adjustment of the  $p\text{H}$  for the zinc sulfide precipitation was made by first adding citric acid, then ammonium hydroxide to the first color change of the indicator. In this manner the indicator color changed so gradually that it was difficult to arrive at a definite  $p\text{H}$ . Fales and Ware (13) found that during the precipitation of zinc sulfide the  $p\text{H}$  should be maintained between 2 and 3 for the best results, and a special buffer solution was added for maintaining the proper  $p\text{H}$ . The Associate Referee found by experiment that adding ammonia to the first color change of bromophenol blue, then adding 2 grams of citric acid produced a solution of the proper  $p\text{H}$  in a volume of approximately 100 ml. Some of the collaborators (Cowling, Hardin, and Hammond) remarked that they were not certain of the proper indicator color in this step. The solution should not be brought to the distinct blue color of the indicator but rather to the color just preceding the distinct blue, which is a green or brownish color. After the citric acid has been added and stirred well, the color should change back to yellow. The method was checked on solutions containing 0.1000 gram of zinc, and complete precipitation was obtained. The addition of other buffering agents is therefore unnecessary, and the specially prepared formic mixture prescribed by Fales and Ware (13) is omitted. The wash solution was also changed from formic acid to citric acid so as to use the same reagent.

Results of from 0.20 to 2.00 per cent of zinc are mostly high and indicate

contamination or incomplete ignition. Contamination may be minimized by proper adjustment of the pH and thorough washing of the precipitate and filter. Scott (8) recommends 900° C. for igniting zinc sulfide to zinc oxide. Some zinc sulfate may be formed by oxidation of zinc sulfide. Hillebrand and Lundell (11) say that zinc sulfate dissociation is complete at 950° C. and that at 1000° C. zinc oxide becomes slightly volatile. Investigation will be made to see whether ignition between 950° C. and 1000° C. gives better results. This method may be made quite accurate for samples of 0.20 per cent or more of zinc, but for low-analysis samples another method may be required.

The Associate Referee treated solutions obtained by separating copper by the methods previously mentioned by the gravimetric method for zinc, but in none of these cases were the results for zinc as consistently near the theoretical as those obtained by the method submitted.

#### VOLUMETRIC METHODS FOR ZINC

As volumetric methods for zinc appeared to be somewhat shorter than gravimetric methods they were also investigated. The method given by Treadwell-Hall (12) based on the procedure used in the laboratories of the New Jersey Zinc Company at Palmerton, Pa., was considered, but the method applies to higher percentages of zinc than are generally found in fertilizers. Titration is with potassium ferrocyanide, and the end point is a change in color from a blue to a pea green. In titrating standard solutions of zinc corresponding to the amounts that are generally obtained from 2 grams of fertilizer, the Associate Referee was unable to obtain satisfactory results with this method.

The method proposed by Cone and Cady (14) and slightly modified by Kolthoff (15) specifying diphenylamine for an internal indicator, is also written for more zinc than is commonly found in 2 grams of fertilizer. Scott (9) gives a method for 1–50 mg. of zinc that specifies diphenylamine as internal indicator for titration with potassium ferrocyanide. The Associate Referee was unsuccessful in the titration of the chloride solution of zinc as the method specified but had more success when the solution was changed to one of sulfuric acid, as in the method submitted.

Seth Walker reported that he obtained good duplication when he titrated the standard zinc solution but that on the sample solutions the duplicates were extremely variable. K. T. Holley commented that experience seems to be necessary in obtaining reproducible values for zinc by either of the two methods submitted. G. S. Fraps called attention to the length of the methods.

#### COLORIMETRIC METHOD FOR ZINC

The Associate Referee for Zinc in Fertilizers is indebted to Hale Cowl-  
ing, Associate Referee for Zinc in Plants, for his valuable collaborative

work. The 1939 Report of the Committee on Recommendations of Referees requested "closer coordination and cooperation between referees preparatory to studies for the determination of the same constituent in different products." The dithizone method of Cowling and Miller, being proposed for determining zinc in plants, was tried by Cowling and the Associate Referee on the 1940 collaborative fertilizer samples. The results are shown in Table 5.

TABLE 5.—Results obtained by the dithizone method of Cowling and Miller

SAMPLE NO.	0	1	2	3	4
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Hale Cowling	0.0097	0.113	0.218	0.527	2.11
W. Y. Gary	0.009	0.110	0.205	0.48	—
Average	0.0093	0.111	0.211	0.503	2.11
Theoretical		0.116	0.215	0.515	2.006

Table 5 indicates that the method gives satisfactory results to approximately 0.5 per cent of zinc and is worth submitting to the fertilizer collaborators. The method requires quite extensive preparations of the reagents but they may be made up in large quantities for numerous determinations. Cowling suggested that the sample solutions may be prepared as directed in the first paragraph of the 1940 collaborative method for copper and zinc in fertilizers, then diluted in 100 ml. volumetric flasks, and aliquots taken for the determination. From this point Cowling said that the time required had been found to be about 30 minutes for each sample when 6-8 determinations are carried simultaneously through the procedure.

The dithizone method of Holland and Ritchie (16) for the determination of zinc in foodstuffs, submitted to collaborators by W. S. Ritchie, Associate Referee, was also tried on the fertilizer samples. The method was found to be adaptable to the analysis of fertilizers low in zinc, but the method for zinc in plants gave better results.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the volumetric method for the determination of copper, adopted as tentative last year (3), be revised as submitted to the collaborators this year and remain tentative.

(2) That the colorimetric method for the determination of copper submitted to the collaborators this year be further studied.

(3) That the gravimetric method for the determination of zinc sub-

\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 46 (1941).

mitted to the collaborators this year be adopted as tentative for samples containing more than 0.20 per cent of zinc.

(4) That the volumetric method for the determination of zinc submitted to the collaborators this year be further studied.

(5) That the dithizone method for the determination of zinc proposed by the Associate Referee on Zinc in Plants be studied collaboratively for the determination of zinc in fertilizers.

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### MONDAY—AFTERNOON SESSION REPORT ON EGGS AND EGG PRODUCTS

By E. O. HAENNI (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The report of the Associate Referee on Unsaponifiable Constituents and Fat is included in this report. Last year he, as Referee, pointed out the urgent need for a method for estimating the egg content of foods, and in a contributed paper, *This Journal*, **24**, 119 (1941), he presented the results of several years' work on the development of a method for the determination of sterol (as cholesterol) in eggs, alimentary pastes, and farinaceous ingredients of alimentary pastes. The Referee also expects to extend this method to the estimation of the egg content of other food products. These results show that the sterol content is a useful index of the egg content of



noodles, and the procedure described gave such satisfactory results in the hands of the Associate Referee that it was submitted to collaborative study.

A sample of spray-dried egg yolk was sent to a number of collaborators with the request that they determine the unsaponifiable matter and cholesterol as directed in the method. Nearly all the collaborators obtained good checks on the sterol determinations, but there was a considerable range in the results of different analysts. This was surprising in view of the fact that the same collaborators obtained quite satisfactory results with a very similar procedure applied to a sample of egg noodles. These results will be presented in the report of the Associate Referee on Sterols in Cereal Foods. To insure that the prepared sample of dried yolk was homogeneous, the Associate Referee determined the sterol content on eight subdivisions. The results obtained were 2.81, 2.80, 2.82, 2.83, 2.80, 2.81, 2.79, and 2.80 per cent (dry basis). It is apparent from correspondence with some of the collaborators that there are some details of the method that require clearer definition. Accordingly publication of the collaborators' results would serve no useful purpose. The Associate Referee will determine those features of the procedure that need further elaboration and submit more specific directions for future collaborative work.

No work was done this year on the determination of fat in eggs.

The Associate Referee thanks the following members of the Food and Drug Administration for their cooperation: S. Alfend, St. Louis; F. J. McNall, Cincinnati; G. Kirsten, New York; J. A. Schuldiner, New York; H. W. Gerritz, San Francisco.

#### DETECTION OF DECOMPOSITION

L. C. Mitchell, Associate Referee, and W. Horwitz present a progress report on chemical methods for the detection of decomposition. A new method for volatile bases is proposed to replace the present tentative aeration method and the absorption method for ammonia nitrogen. The absorption method has been studied by previous associate referees as a rapid and convenient procedure for duplicating the results obtained by the more tedious and complicated aeration method of years' standing. The associate referee points out that the fixed alkali used in the former methods may possibly attack the proteins to some extent, with progressive liberation of volatile bases. The proposed method, providing for removal of the proteins by precipitation with salt and alcohol before alkali is added, represents a commendable new departure having a sound theoretical basis. However, the Referee believes that the new method should be demonstrated to give results of practical significance comparable to or better than the older methods before the latter are abandoned.

There is a particular need for chemical methods for detecting decomposition based on non-volatile constituents in dried eggs since organoleptic

methods are not suitable. The acidity of the ether extract has long been used as one criterion of decomposition. The present official method is rather inconvenient, and a rapid method applicable to liquid eggs was adopted tentatively in 1938. A rapid method was also recommended for adoption tentatively in 1932 by Associate Referee Grigsby, *This Journal*, 15, 344 (1932), but the Association did not approve because the method was applicable only in the case of dried eggs. This useful rapid method was then evidently overlooked by subsequent associate referees. The Referee suggests that it be given further consideration for adoption as a tentative method.

No report on the determination of added glycerol in eggs was submitted.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the associate referee determine the details of the methods for unsaponifiable matter and cholesterol that need clearer definition and submit the methods to collaborative study with the more specific directions.

(2) That study of the application of the methods to food products containing eggs be continued.

(3) That study of the method for the determination of fat by acid hydrolysis be continued.

(4) That the rapid method for the determination of acidity of ether extract in dried eggs, *This Journal*, 15, 341 (1932), be considered by the Associate referee for recommendation for adoption next year.

(5) That study of chemical methods for the detection of decomposition be continued.

(6) That study of methods for the determination of added glycerol be continued.

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For report on unsaponifiable constituents and fat see previous report of the Referee on Eggs and Egg Products.

#### REPORT ON DETECTION OF DECOMPOSITION IN EGGS

By L. C. MITCHELL, *Associate Referee*, and WILLIAM HORWITZ  
(U. S. Food and Drug Administration, Minneapolis, Minn.)

This is a progress report of the work started last year in the development of chemical methods that may be of value in the detection and measurement of decomposition in eggs.

The present tentative method for the determination of ammonia nitrogen has been questioned, as evidenced by the work of previous associate referees, *This Journal*, 20, 159 (1937); 21, 179 (1938); 22, 298 (1939), and

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 57 (1941).

the method of Bandemer and Schaible<sup>1</sup> was proposed. Schaible, however, in a private communication, called attention to the exacting conditions under which this method must be carried out. In both of these methods, the volatile bases, erroneously designated as ammonia nitrogen, are liberated by sodium or potassium carbonate, substances that may have some hydrolytic action on proteins, even at room or somewhat higher temperatures, possibly producing progressive deamination. Since the volatile bases are liberated by some fixed alkali before their estimation, and as fixed alkalis attack proteins to some extent it seems essential that any method for the estimation of volatile bases should provide for the removal of proteins before their liberation and estimation. Accordingly, the method now proposed for the attention of this Association provides for the removal of the proteins before the estimation of the volatile bases.

In passing, attention of this Association should possibly be called to the fact that the present official method for the determination of acidity of ether extract, *Methods of Analysis, A.O.A.C.*, 1940, 314, 22, does not provide for the liberation of the non-volatile fatty acids from their salts before extraction with ether. In the detection of certain types of spoilage in many samples of dried whole eggs or dried yolks this omission may be significant.

For the consideration of this Association the methods developed to date (May, 1940) include the determination of volatile acids, which is a modification of the Clark and Hillig method, *This Journal*, 21, 694 (1938); the determination of volatile bases, which is a new procedure for eggs; and the determination of lipids and lipid  $P_2O_5$ , which is the present tentative method for lipid and lipid  $P_2O_5$ , *Methods of Analysis, A.O.A.C.*, 1940, 310, 10. In addition some work was done to adapt the Van Slyke method to the estimation of amino acids, and the alkaloidal procedure (chloroform or ether extraction from acid, then alkaline solution) to the estimation of the non-volatile acids and bases formed during putrefaction of eggs, but this work has not progressed sufficiently to report at this time. Further, the direct titration of the egg material in alcohol or formaldehyde should theoretically give the total acids, whether free or combined, but thus far indicator difficulties have not been overcome.

The details of the methods are given as follows:

#### VOLATILE ACIDS

(Examine samples for decomposition without delay.)

#### 1

##### APPARATUS

For convenience in operating the Clark and Hillig steam distillation assembly described in *This Journal*, 21, 685 (1938), insert through the stopper of the boiler a short glass tube provided with a short rubber tubing, a pinchcock, and another short glass tube bent at right angle to conduct the steam away from the operator. In addition, insert into the safety tube a small glass tube attached by means of rubber tubing to a 250 ml. globe-shaped separatory funnel to conduct water drop-

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 8, 201 (1936).

wise into the boiler in order to maintain the volume of water in the boiler at the 1500 ml. level (marked on boiler) throughout the distillation. (Steam is generated by the electrically operated boiler, and accurately and smoothly controlled by means of the rheostat. The specified volume in the distillation flask is maintained by means of a suitably adjusted small flame.)

## 2

## REAGENTS

(a) *Water*.—Use boiled distilled water.

(b) *2 N sulfuric acid solution*.—Add (with care) 55 ml. of  $\text{H}_2\text{SO}_4$  to water, and dilute to 1 liter.

(c) *Phosphotungstic acid solution*.—Dissolve 20 grams of phosphotungstic acid in water, and dilute to 100 ml.

(d) *Sulfuric acid solution (1+1)*.—Add (with care) 10 ml. of  $\text{H}_2\text{SO}_4$  to 10 ml. of water.

(e) *Magnesium sulfate*.— $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  crystals.

(f) *Standard sodium hydroxide solution*.—Prepare a 0.02 N solution by diluting a 0.1 N solution that has been standardized by titration against Bureau of Standards acid potassium phthalate. (Such alkali solutions are stored preferably in heavily paraffined bottles, or in bottles coated with rubber solution known as "Goodrich Vulcalock Cement," and protected from air ( $\text{CO}_2$ ) by a device similar to the one shown in *This Journal*, 21, 686 (1938).

(g) *Phenolphthalein indicator*.—Dissolve 1 gram of phenolphthalein in 100 ml. of 95% alcohol.

## 3

## PREPARATION OF SOLUTION

*Liquid eggs*.—Weigh accurately, by difference, into a 500 ml. volumetric flask containing 300–350 ml. of water, approximately 50 grams of the well-mixed sample, and mix. Add with continuous mixing 15 ml. of 2 N  $\text{H}_2\text{SO}_4$ , then 30 ml. of the phosphotungstic acid solution, and allow to stand about 10 minutes. If no clear separation occurs, add with continuous mixing 5 ml. more of the phosphotungstic acid solution and allow to stand again about 10 minutes. If necessary, repeat such operation until a clear separation occurs, avoiding any large excess of reagent. Make to 500 ml. with water, shake vigorously, and filter through an 18½ cm. folded filter, covering the filter with a watch-glass during filtration.

## 4

## DETERMINATION

Transfer 150 ml. of the clear filtrate, 3, to the distillation flask of the distillation assembly, 1, containing 25 grams of the  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; add 1 ml. of  $\text{H}_2\text{SO}_4(1+1)$ ; connect assembly and distil 100 ml. with just sufficient steam being generated in the boiler to prevent distillate from entering the steam delivery tube from the boiler. Then steam distil at the maximum speed of the assembly, maintaining the volume in the distillation flask at the 50 ml. level (marked on the flask) until five more 100 ml. portions have been collected. Combine all six 100 ml. portions, and titrate with standard alkali, using phenolphthalein indicator. Subtract the titration blank, and report the volatile acids in terms of ml. of 0.1 N per 100 grams of sample.

**NOTES.**—This procedure will not determine those fatty acids that are volatile with steam, but are not soluble in slightly acid solution.

An occasional sample of decomposed eggs having a distinct to strong ammoniacal odor may require an additional 5 ml. of the 2 N  $\text{H}_2\text{SO}_4$  in order to yield a clear filtrate. The precaution, however, is not included in the method as such eggs are seldom in need of chemical examination.

As fresh eggs have no volatile acids, at least within the experimental error of blanks, this method of determination is promising for the detection

of incipient protein and/or lipid decomposition. The experimental work completed shows that formic acid, the most difficult of the volatile fatty acids to steam distil, is quantitatively recovered under the conditions given in the method. The method makes no attempt, however, to identify the various volatile acids produced during spoilage. Formic, acetic, propionic, butyric, iso-butyric, valeric, and iso-valeric acids have been found in decomposed yolks by the curve method of Clark and Hillig, *This Journal*, 21, 684, 688 (1938).

Volatile bases, including ammonia, can not be determined in the above prepared solution, as the protein precipitants also precipitate partially or wholly, the nitrogenous bases.

#### VOLATILE BASES

5

##### APPARATUS

Same as specified for volatile acids.

6

##### REAGENTS

(a) *Water*.—Same as 2(a).

(b) *Salt solution*.—Dissolve 25 grams of NaCl in water and dilute to 500 ml.

(c) *Sulfuric acid solution*.—Same as 2(d).

(d) *Milk of lime*.—Ignite lime or  $\text{CaCO}_3$  to CaO, cool, transfer with continuous mixing 40 grams to a liter flask containing about 500 ml. of water, and dilute to 1 liter. Shake well before using.

(e) *Standard sodium hydroxide solution*.—Same as 2(f).

(f) *Standard acid solution*.—Standardize 0.1 N HCl or  $\text{H}_2\text{SO}_4$  solution by titration against 0.1 N alkali, using phenolphthalein as indicator.

(g) *Methyl red indicator*.—Dissolve 0.1 gram of methyl red in 100 ml. of 95% alcohol (warm), and filter, if necessary.

7

##### PREPARATION OF SOLUTION

(a) *Liquid eggs*.—Weigh accurately, by difference, approximately 25 grams of the well-mixed sample into a 250 ml. volumetric flask containing 50 ml. of the NaCl solution and 15 ml. of the 2 N  $\text{H}_2\text{SO}_4$ , and mix. If the solution is not acid to congo paper, add 5 ml. more of the  $\text{H}_2\text{SO}_4$ , and mix. Add with continuous mixing 130 ml. of 95% alcohol. Allow to stand a few minutes with occasional rotation of flask for gas bubbles to rise to surface, cool to room temperature, and fill to mark with water (dispersing foam, if any, by addition of 1–2 drops of ether); shake vigorously, and filter through an 18½ cm. folded filter, covering the filter with a watch-glass during filtration.

(b) To correct for the error due the volume occupied by the precipitate, particularly in samples of yolks, repeat the determination, weighing the same amount of sample into a 500 ml. volumetric flask containing 100 ml. of the NaCl solution and 15 ml. of the  $\text{H}_2\text{SO}_4$  (with precaution noted above regarding acidity), and mix. Add with continuous mixing 260 ml. of 95% alcohol, and proceed as directed under 7(a).

8

##### DETERMINATION

(a) Transfer 50 ml. of the clear filtrate, 7(a), or 100 ml. of 7(b), to the distillation flask of the distillation assembly, 1, add 2–3 drops of phenolphthalein indicator, then add 10 ml. of the milk of lime (or sufficient quantity to render slightly alkaline) by means of a rapidly flowing (transfer) pipet in such manner that the reagent flows to the bottom of flask, and connect assembly. Distil at first slowly, then at the

maximum speed of the assembly, maintaining the volume at 50 ml. level (marked on flask) until approximately 300 ml. has been collected (if excessive foaming develops, control by adjusting rheostat), taking care that the tip of the adapter extends below the surface of the measured amount of standard acid in the receiving flask. Titrate with standard alkali, using methyl red indicator, and report as ml. of 0.1 *N* per 100 grams of sample.

To correct for the error due to volume occupied by the precipitate, multiply the result obtained on the 500 ml. determination by two and subtract the result obtained on the 250 ml. determination, and report as ml. of 0.1 *N* per 100 grams of sample (corrected).

NOTES.—Amines, as well as ammonia, are putrefactive products of proteins.<sup>2</sup> On evaporating the neutralized distillate to dryness and adding excess NaOH solution the fish-like odor of the lower amines has been noticed repeatedly in the work on yolks. Further, microchemical tests<sup>3</sup> with gold hydrogen bromide in sirupy  $H_3PO_4$  on the residue obtained by distilling into slight excess HCl solution and evaporating the distillate to dryness have shown crystals other than those of ammonia. For these reasons, the method is designated "volatile bases" instead of by the erroneous name of "ammonia nitrogen."

The combination of salt, acid, and alcohol yields a clear filtrate, apparently free from proteins and lipids. As fresh eggs have but a small amount of volatile bases, this method of determining them offers promise in detecting incipient protein and possibly lecithin decomposition. The experimental work thus far completed shows that added ammonia is quantitatively recovered under the conditions given in the method. No work has been done on the recovery of known amounts of the volatile amines.

If a weak alkali is required in ammonia distillations, magnesium or calcium hydroxide is ordinarily used. In this work it was found that much foaming, especially after the alcohol has distilled off, is produced by magnesium hydroxide, the weaker of the two alkalis. On the other hand, the foaming, if any, that is caused by calcium hydroxide is readily controlled by adjusting the rheostat. This steam distillation method, with milk of lime, has been used successfully on a number of products where the usual distillation methods were found unsatisfactory because of excessive foaming.

## 9

## LIPIDS AND LIPID P.O.

Determine as directed under *Methods of Analysis*, A.O.A.C., 1940, 310, 10.

NOTES.—See *This Journal*, 16, 304 (1933) for various ways to transfer aliquots for the lipid work. Pipetting without filtering is the preferred method of transferring an aliquot. Recently George E. Keppel, Minneapolis Station, devised a filtering gadget that is placed over the tip of the pipet. It consists of a small hollow cylinder of 60–80-mesh wire gauze with a short piece of pressure tubing at one end to accommodate the pipet tip and closed at the lower end. The gauze is cut L-shape, and the narrow projection is rolled tightly to form the closed (lower) end around which the cylinder is formed. A short piece of pressure rubber tubing is inserted into the open end, and both ends are wrapped with several turns of thin copper wire. Synthetic rubber is preferable to rubber as it is less likely to be affected by the solvent.

<sup>2</sup> R. A. Gortner, *Outlines of Biochemistry*, 2nd Ed. (1938), pp. 549–550; R. E. Buchanan and Ellis I. Fulner, *Physiology and Biochemistry of Bacteria*, Vol. III, pp. 370–372; Carl L. A. Schmidt, *The Chemistry of the Amino Acids and Proteins*, 1938 Ed., p. 238, Henry Gilman, *Organic Chemistry*, Vol. II, (1938), p. 1099.

<sup>3</sup> Charles C. Fulton, *Am. J. Pharm.*, 112, 51, 134 (1940).

The weight of sample used should be sufficient to yield 0.2–0.4 gram of lipids in the aliquot taken for analysis. The volume of mixed solvent should be at least nine times the weight of the sample (about 13 ml. of water is miscible in 100 ml. of the mixed solvent) in order to assure a clear liquid after separation of the proteins. The amount of molybdate solution to be used may be calculated from the weight of lipids found, as the  $P_2O_5$  amounts to about 3% of the lipids from fresh eggs. Add an excess of 10–15 ml. of molybdate solution after allowing 1 ml. for each mg. of  $P_2O_5$ . Paper is preferable to cotton for filtering in the purification of lipids from decomposed eggs.

There appears to be no relation between the acidity of lipids extracted by the mixed solvent and the decomposition of the lecithin as shown by the decreased content of  $P_2O_5$  in the lipids. The acidity was determined not only on an aliquot directly but also on another aliquot after purification of the lipids. Possibly the lack of relationship is due to the fact that no provision is at present made in the method to liberate the acids which were produced and neutralized in the process of spoilage. For these reasons no data are included in the tables on the acidity of lipids.

Table 1 gives some results obtained by the above methods on extremely decomposed eggs that had stood several months at room temperature, and

TABLE 1.—*Comparative results on eggs in extreme stages of decomposition and on fresh eggs*

SAMPLE NUMBER	VOLATILE ACIDS	VOLATILE BASES	LIPIDS	LIPID $P_2O_5$
0.1 N/100 g.				
		ml.	per cent	mg./g. lipid
Decomposed				
Yolks				
629	94	514	34.89	17.9
447	98	76	32.85	3.6
625	127	319	35.34	16.3
502	18	325	36.25	21.7
508	33	697	31.08	0.7
154	187	1024	33.08	0.2
630	603	1121	35.54	19.1
Decomposed				
Whites				
195*	0.9	10.3		
448†	31.6	23.2		
445‡	43.4	349.6		
629‡	200.4	335.0		
Fresh				
Yolks	0.0	7.6	33.82	27.7
Yolks	0.0	9.6	34.13	28.1
Whites	0.0	3.3	—	—
Whole	0.0	7.1	13.38	27.7

\* Although this sample was subjected to the same conditions as the others, organoleptic analysis indicated very little decomposition, and the above results confirmed this.

† Typical.

‡ Extreme.

that are not likely to be encountered in commercial channels, and on fresh eggs. Of the large number of decomposed eggs examined, 7 yolks and 4 whites were selected for this table, not only to show the enormous range of values that were encountered but also to show the lack of correlation between the different types of decomposition. Such results indicate that it is not likely that any one chemical method can be developed to detect and evaluate spoilage in eggs, a conclusion that appears obvious in view of the limited well-defined biochemical task of the microbial species.

Table 2 gives a few duplicate results obtained on eggs having varying amounts of volatile acids and bases. Table 3 gives results on the recovery of known amounts of ammonia or formic acid added to fresh eggs.

TABLE 2.—*Typical results of duplicate determinations of volatile acids and bases*

ml. 0.1 N/100 g.				
Volatile	697.1	349.5	23.0	9.7
Bases	698.0	350.0	23.2	8.9
Volatile	20.35	4.2	1.0	0.0
Acids	20.40	4.2	0.8	0.3

TABLE 3.—*Recoveries of added  $\text{NH}_3$  and  $\text{HCOOH}$  by the methods for volatile acids and bases*

SUBSTANCE ADDED	AMOUNT ADDED, 0.1 N	AMOUNT RECOVERED 0.1 N	
		ml.	per cent
$\text{NH}_4\text{Cl}^*$	20.00	20.08	100.4
	20.00	19.94	99.7
	7.50	7.50	100.0
	4.00	4.01†	100.3
$(\text{NH}_4)_2\text{HPO}_4^*$	18.30	18.14	99.1
	18.30	18.28	99.9
$\text{HCOOH}^\dagger$	20.46	20.33	99.4
	19.70	20.08	101.9
	9.85	10.03	101.8
	4.00	4.14‡	103.5

\* Results corrected for volume of precipitate.

† Results not corrected for volume of precipitate, following the original method of Clark & Hillig.

‡ 0.02 N base used for back titration.

### RECOMMENDATIONS\*

It is recommended—

(1) That the work on the development of chemical methods for the detection and measurement of spoilage in eggs be continued.

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 57 (1941).



(2) That studies on methods for the determination of added glycerol be continued.

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No report on added glycerol was given by the associate referee.

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## REPORT ON PRESERVATIVES

By WILLIAM F. REINDOLLAR (State of Maryland Department of Health, Baltimore, Md.), *Referee*

The work this year was devoted to an extension of the qualitative test for saccharin to baked goods, and a collaborative study of the method for the several types of foods to which it has proved to be applicable. As benzaldehyde has been reported as a possible interfering substance, the test was applied to a cherry soda as purchased and to one containing a substantial amount of added benzaldehyde. In neither case was there sufficient color developed by this compound to justify the inclusion of special directions for its removal. Vanillin, which definitely does interfere, is removed with petroleum benzin rather than with carbon tetrachloride, as was directed last year.

Paired samples, one containing added saccharin and the other none, of ginger ale, apple butter, catsup, India relish, and butter cookies, were prepared and submitted to the collaborators along with the following directions.

### METHOD

#### REAGENTS

(a) *Ethereal solvent*.—Mix equal volumes of ethyl ether and petroleum benzin (b.p. 30–60°).

(b) *Phenol-sulfuric acid*.—Dissolve pure colorless crystalline phenol in an equal weight of  $H_2SO_4$ .

(c) *Petroleum benzin* (b.p. 30–60°).

Sea sand, washed and ignited.

#### PREPARATION OF SAMPLE

(a) *Non-alcoholic beverages*.—Add 3 ml. of HCl to 25 ml. of sample contained in a separatory funnel. If vanillin is present, remove it by extracting with several portions of petroleum benzin. Discard the petroleum benzin. Extract with 50, 25, and 25 ml. of the ethereal solvent. Wash the combined ethereal extracts once with 5 ml. of water, remove the major portion of the solvent, transfer to a 30 ml. beaker, and allow to dry at room temperature.

(b) *Semi-solid preparations*.—Transfer 25 grams of the sample to a 100 ml. volumetric flask with a small quantity of hot water and add sufficient boiling water to make a volume of about 75 ml. Allow the mixture to stand for an hour, shaking occasionally. Then add 3 ml. of glacial acetic acid, mix thoroughly, add a slight excess (5 ml.) of 20% neutral Pb acetate solution, dilute to the mark with cold water,

mix, allow to stand for 20 min., and filter. Transfer 60 ml. or more of the filtrate to a separatory funnel and proceed as directed under (a).

(c) *Baked goods*.—Grind 25 grams of the sample, mix thoroughly with 50 grams of the sea sand, and extract with petroleum benzin in a Soxhlet until approximately fat free (1–2 hours). Transfer the extracted mass to a 300 ml. Erlenmeyer flask, add 100 ml. of alcohol, and reflux on a boiling water bath for 30 min., shaking frequently. Filter through a Büchner containing a No. 2, 7 cm. filter paper wet with alcohol. Transfer the alcoholic filtrate to a 100 ml. beaker, evaporate to  $\frac{1}{2}$  volume, add 50 ml. of water and sufficient 10%  $\text{Na}_2\text{CO}_3$  solution to make alkaline, and evaporate to 50 ml. Transfer the aqueous solution to a separatory funnel and proceed as directed under (a).

#### DETERMINATION

Add 5 ml. of the phenol- $\text{H}_2\text{SO}_4$  reagent to the residue remaining after the evaporation of solvent and heat for 2 hours at 135–140° C. Cool, dissolve in small quantity of hot  $\text{H}_2\text{O}$ , and pour into about 250 ml. of water. Add a small quantity of filter-cel, allow to stand 3 hours or overnight, and filter. Make alkaline with 10%  $\text{NaOH}$  solution and dilute to 500 ml. A magenta or reddish purple color develops if saccharin is present. A yellow, buff, or pale salmon shade is not significant.

All the collaborators reporting were able to properly identify the samples, and no one reported experiencing any difficulty with the method. The results appear in the table.

<i>Collaborator</i>	<i>Sample A—Saccharin</i>	<i>Sample B—No Saccharin</i>
F. J. McNall	Positive	Negative
H. I. Macomber	Positive	Negative
L. M. Beacham	Positive (catsup only)	
M. S. Oakley	Positive	Negative
D. M. Oberseider	Positive	Negative
Referee	Positive	Negative

It is recommended\* that the qualitative test for saccharin in non-alcoholic beverages, semi-solid preparations, and baked goods be adopted as a tentative method.

#### REPORT ON BENZOATE OF SODA

By A. E. Mix (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Some work was done on the modified Monier-Williams method. The results obtained are in keeping with those reported at the 1938 meeting, and no report is offered at this time.

The Associate Referee has worked on a new method, which appears very promising, but not enough work has been done to warrant any recommendation. It is recommended that study of the determination of small quantities of sodium benzoate be continued.

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 61 (1941).

## REPORT OF COLORING MATTERS IN FOODS

By C. F. JABLONSKI (U. S. Food and Drug Administration, New York City), *Referee*

The Committee on Recommendations requested the Referee to continue the collaborative investigation of the quantitative estimation of Ponceau SX (FD & C Red No. 4) in presence of Ponceau 3R (FD & C Red No. 1). With this purpose in view the Referee sent out to the collaborators seven sets of samples, consisting of seven subdivisions each, with instructions to estimate the dye mixtures by a submitted method.

The samples were of the following compositions (based on  $\text{TiCl}_3$  titrations):

SAMPLE	PONCEAU SX per cent	PONCEAU 3R per cent	TOTAL COLOR per cent
1	87.37	1.13	88.50
2	0.00	90.35	90.35
3	44.24	45.18	89.42
4	88.48	0.00	88.48
5	1.11	89.22	90.33
6	22.12	67.76	89.88
7	66.36	22.56	88.92

The reports of the collaborators as received, follow:

*O. L. Evenson, Food and Drug Adm., Washington, D.C.*

SAMPLE	PONCEAU SX per cent	PONCEAU 3R per cent	TOTAL COLOR per cent
1	88.2	0.6	88.8
2	1.8	88.5	90.3
3	43.8	45.4	89.2
4	88.5	0.9	89.4
5	3.0	87.6	90.6
6	21.6	69.5	91.1
7	64.5	24.0	88.5

*S. S. Forrest, Food and Drug Adm., Washington, D.C.*

1	88.8	1.2	90.0
2	1.8	87.8	89.6
3	42.0	46.9	88.9
4	89.0	0.0	89.0
5	3.0	88.3	91.3
6	22.2	68.9	91.1
7	64.8	22.9	87.7

*S. H. Newburger, Food and Drug Adm., Washington, D.C.*

1	87.0	0.93	87.93
2	0.6	87.6	88.2
4	89.4	0.0	89.4

*R. W. Stewart, Food and Drug Adm., Washington, D.C.*

1	87.8	0.0	87.8
2	8.5	77.5	86.0
3	41.7	44.5	86.2
4	88.8	0.00	88.8
5	0.9	88.8	89.7
6	24.0	65.8	89.8
7	64.8	23.8	88.6

*L. Koch, H. Kohnstamm & Co., Brooklyn, N.Y.*

1	88.20	0.00	88.20
2	0.00	90.50	90.50
3	42.32	46.96	89.28
4	89.00	0.00	89.00
5	1.65	88.20	89.85
6	20.70	70.50	91.20
7	66.00	23.50	89.50

*J. J. Morris, H. Kohnstamm & Co., Brooklyn, N.Y.*

1	87.95	0.62	88.57
2	0.00	90.37	90.37
3	41.72	47.02	88.74
4	89.44	0.00	89.44
5	1.68	88.25	89.93
6	19.51	69.53	89.04
7	66.93	22.98	89.91

*I. Hanig, H. Kohnstamm & Co., Brooklyn, N.Y.*

1	87.64	0.00	87.64
2	0.00	89.45	89.45
3	45.80	43.08	88.88
4	90.05	0.00	90.05
5	1.50	87.88	89.38
6	20.11	71.50	91.61
7	65.43	23.05	88.48

*J. L. Hogan, Food and Drug Adm., New York*

1	88.25	0.20	88.45
2	0.00	88.80	88.80
3	41.40	47.30	88.70
4	89.45	0.00	89.45
5	1.20	89.05	90.25
6	21.60	70.50	92.10
7	66.65	22.60	89.25

The collaborators offered the following comments and criticism:

*O. L. Evenson.*—In some cases the titrations on the mixtures varied as much as 0.3 ml.  $\text{TiCl}_3$ . After treatment the titrations checked within 0.1 ml.  $\text{TiCl}_3$ .

*S. S. Forrest.*—The various analyses of these samples checked quite closely, within 0.1 or 0.2 ml. per 0.2 gram of color.

*S. H. Newburger.*—After oxidation the corrected titration value of Sample 4 was greater than the original titration.

*R. W. Stewart.*—It has been the experience of the members in this laboratory that the end point obtained when titrating Ponceau SX with sodium citrate as a buffer is very hard to discern and that the results obtained with this buffer are usually high. The above is probably one explanation why it is so difficult not to overtitrate the mixtures of Ponceau SX and Ponceau 3R. In the case of Mixtures 1 and 4, a larger titration was obtained after treatment than before. The same solutions of hydrogen peroxide and sodium arsenite were used by several collaborators in this laboratory and, as far as I know, I was the only one to obtain such results, and I am unable to explain the discrepancy.

*L. Koch.*—Experimental titrations indicated that the use of tartaric acid was not necessary because of the acidic nature of the sodium bitartrate.

The other collaborators made no comments.

### DISCUSSIONS

The comments and criticism received from several of the collaborators indicate an unexpected difficulty in obtaining a satisfactory end point by using sodium citrate as a buffer agent. While the greater part of the results submitted can be considered as satisfactory, there are some that show a considerable deviation from the theoretical values. Whether these discrepancies are caused by the use of said buffer or are the result of other factors can not be answered without further investigation.

It is the Referee's experience, however, that under given conditions no difficulties are encountered in obtaining a sharp end point with the sodium citrate buffer.

It is therefore the Referee's opinion that these minor difficulties should be investigated and collaborative work repeated next year in order to conclude this problem.

### RECOMMENDATIONS\*

It is recommended—

(1) That collaborative work be continued on the method for the quantitative determination of FD & C Red No. 4 (Ponceau SX) in presence of FD & C Red No. 1 (Ponceau 3R).

(2) That investigational work be continued on the quantitative separation and estimation of FD & C Yellow No. 5 (tartrazine) and FD & C Yellow No. 6 (Sunset Yellow FCF.).

(3) That investigational work be undertaken to separate and determine quantitatively mixtures of FD & C Green No. 2 (light green SF yellowish), FD & C Green No. 3 (Fast Green FCF.), and FD & C Blue No. 1 (Brilliant Blue FCF.).

(4) That collaborative work on analytical methods for the coal-tar colors certifiable for use in foods be conducted.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 61 (1941).

## REPORT ON METALS IN FOODS

By H. J. WICHMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

## ARSENIC AND ANTIMONY

Fourteen collaborators obtained recoveries of 92–108 per cent on the known arsenic solutions sent out by the associate referee. The results seem to fluctuate around the mean in the arsenic determinations on the shrimp and tobacco even more widely. The Referee is unable to account satisfactorily for the high results, but surmises that they are due to varying blanks in the determination of the smaller quantities of arsenic. Blanks become insignificant with the larger amounts. Low recoveries may be due to a variety of causes, one of which is incomplete evolution. To drive 500 micrograms of  $\text{As}_2\text{O}_3$  into the receiver in 5 minutes requires active zinc and the vigorous application of heat. If time is of no particular object, the reaction may be conducted more leisurely. However, Cassil, *This Journal*, 24, 196 (1941), has shown that as large a quantity as 10 mg. of  $\text{As}_2\text{O}_3$  may be evolved in 5 minutes with no increase of zinc or acid. His evolution curves show that by far the greater part of the arsine is evolved between the first and third minutes. Analysts should exert the maximum driving force behind their evolutions that occasional foaming and the necessity for condensing water and acid vapors will permit. If, in spite of all efforts, incomplete evolutions persist, the Referee believes the only remedy lies in increasing slightly the amounts of acid and zinc and prolonging the evolution several minutes.

Leaky apparatus may cause low results, and there must be considerable gas pressure inside the apparatus. A judicious grinding of the joints with fine emory powder may help. The joints may be lubricated, but the lubricant should never enter the generator to contaminate the surface of the zinc.

A certain relationship should exist between the rate of arsine evolution and the depth of the mercury solution. If the diameters of the leucite and absorption tubes are not well proportioned, some arsine might escape absorption. In such cases the only remedy is to lengthen and narrow the absorption part of the apparatus. The Referee has never been able to detect any arsenic on Gutzeit strips placed in outlets from his absorption tubes even when the hydrogen evolution was at the rate of 300 ml. or more per minute.

Some collaborators seem to have had trouble with dissolving mercury arsenide stains from their leucite tubes. They should be kept well polished on the inside. The Referee seconds the associate referee's suggestion that sufficient time should always be taken to allow any deposit to dissolve before the titration is begun. The oxidation is sometimes sluggish. There may

be other reasons for low recoveries, but the ones discussed are probably the most obvious.

The associate referee's report further indicates that the "scatter" on the arsenic determinations on shrimp and tobacco is too great. Here the problem is two-fold, viz., proper sample preparation and elimination of the errors of the final determination. The associate referee suggests trial of peroxide combustion as against the  $\text{HNO}_3\text{-H}_2\text{SO}_4\text{-HClO}_4$  wet digestion as preparatory methods for shrimp and tobacco. Ashing methods have been suggested in previous reports but have had only limited and unsatisfactory trials. The Referee hopes that the next assistant will have more time to devote to the problems of sample preparation as well as to the actual determination of arsenic in order to complete these phases of the arsenic problem and so provide an opportunity for other related problems.

Nothing was done on the determination of antimony. The Referee hopes that some analyst will soon have the ambition and time to apply the Casil-Wichmann method for arsenic to antimony. All principles of such a method appear to be available.

The Referee does not feel justified in making any recommendations with respect to adoption or rejection of any new arsenic methods at this time. Therefore last year's recommendations are repeated.

#### COPPER

The associate referee has consolidated information obtained by the two previous associate referees into a definite carbamate method for the determination of copper. Two methods of isolation, the sulfide and a dithizone extraction process, were investigated. The results obtained are quite satisfactory, and if they had been available last year, the Referee would unhesitatingly have made a recommendation for tentative adoption in order to include the method in *Methods of Analysis, A.O.A.C.*, 1940. However, there is no such incentive for the adoption of new methods at this time, as another year may see further changes. In the meantime, analysts may follow the method outlined by the Associate Referee on Copper with every assurance of reasonably satisfactory results.

#### FLUORINE

The Associate Referee on Fluorine discussed certain small errors in the fluorine method that had been suspected previously, but not clearly defined. The associate referee described how he determined the presence of a small fluorine blank derived from glassware, but presumably nothing much can be done about it unless fluorine-free glass distillation apparatus becomes available. Small, but unfortunately variable, retentions of fluorine were also discussed.

The Referee calls the attention of analysts to a paper by McClendon and Foster.<sup>1</sup> They describe a closed system of ashing similar to the prepar-

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 13, 280 (1941).

atory stages of an iodine determination, and also a distillation flask, the novel feature of which is the admission of superheated steam at 150° C. from the bottom. It is claimed that a trap prevents practically any sulfuric acid (and presumably no phosphoric acid) from being carried over into the receiver. The authors say that the fluorine is completely volatilized in 100 ml. of distillate. If these claims can be substantiated, possibly a change in apparatus might solve some of the fluorine problems, such as the fluorine blanks, retention of minute quantities of fluorine, and the distillation of undesirable interfering acids or other compounds.

The colorimetric aluminum-aluminon fluorine method described by the associate referee is a useful check on the titration method. Both methods determine the fluorine in the distillates equally well. The average deviation of these methods was not reported, but judged roughly from the data it may be approximately half a microgram. It seems that the average error of a present-day microfluorine determination, including errors of sample preparation, isolation, and determination, is not less than 1 microgram and may be slightly more. Therefore the percentage error depends upon the quantities involved.

The associate referee has listed some of the numerous problems that still exist. The Referee does not feel justified in recommending a microfluorine method for tentative adoption at this time. He recognizes that a fairly accurate, and above all, rapid, fluorine method is urgently needed in the spray residue field. The methods used today for this purpose give fair results, but they do not warrant adoption by this Association.

#### FUMIGATION RESIDUES

Two years ago the Associate Referee on Fumigation Residues was able to determine about 0.5 mg. of hydrocyanic acid, and the smallest detectable end point corresponded to about 0.01 mg. He did this by an aeration method of isolation and titration with silver. This year he determined 10–100 micrograms of hydrocyanic acid with a maximum error of about 12 per cent. This was done on pure substances and without isolation of the cyanide from other materials for the purpose of ascertaining the possibilities of the two colorimetric methods tested in a preliminary way two years ago. The details of an acceptable method of isolation for microgram quantities of hydrocyanic acid will necessarily follow.

The thiocyanate method appears to have the advantage of greater specificity, and also of reproducibility in spite of blank troubles encountered in the sodium sulfide reagent. This one disadvantage should be susceptible to correction. The phenolphthalin method is less specific, very sensitive, but also more erratic; on the other hand, it does not require much blank control. The Association now seems to be in the fortunate position of having promising methods for the determination of both macro and micro quantities of hydrocyanic acid as a fumigation residue in foods.



Sample preparation and isolation should also be investigated further, and collaborative work next year should develop both the micro and macro phases of these determinations to the point where tentative adoption can be considered. The Referee believes that the micro methods described by the associate referee should be of interest to others besides food chemists.

#### LEAD

The associate referee's report refers to modifications of the lead determination on spray residue necessitated by recent changes in tolerance. It does not seem necessary to subject such changes to collaboration, since they may be classified as editorial. The Referee approves of their adoption.

#### SELENIUM

The associate referee made an excellent report on the determination of selenium. Apparently the plus or minus errors of the tentative method, very small in any case, can be prevented entirely by restricting the sample to such a weight that no more than 1000 micrograms of selenium are ever distilled and titrated. The associate referee increased the sensitivity and accuracy of the tentative method in the extremely low, 0-10 microgram, range, not by changing the principles involved but merely by applying micro technic. Deviations of only 0.05 or 0.10 micrograms in a purely chemical method seem remarkably small. A method that determines 1 microgram of selenium with an error of 5-10 per cent is certainly approaching the accomplishments of spectrographic methods.

After setting up a standard for performance in the modified tentative method, the associate referee examined four other methods and evaluated them against it. None of them approached the improved tentative method in value. The two direct colorimetric methods failed because of a lack of either sensitivity or reproducibility. The associate referee intends to test some other methods that have been suggested. There seems to be no reason for recommending any changes at this time.

#### ZINC

The associate referees have for a second time submitted collaborative samples of foods for the determination of zinc. The "scatter" of the results is about the same as last year, and more than desired. The associate referees believe the errors result mostly from incomplete solution of the zinc from the ash by hydrochloric acid and from variable blanks contributed by the glassware. They propose to concentrate on these two points and then develop a photometric "mixed color" method. The Referee endorses the recommendations, but wishes to make a few possibly helpful suggestions.

Some of the reagents used in this method are made up in alkaline solution, notably the dithizone itself, and here may be found a double oppor-

tunity for the extraction of zinc from glass. It might be better to avoid too alkaline a pH in the zinc determination. Fischer has extracted zinc from acetate-buffered solutions, and perhaps the possibilities of extraction from approximately neutral solutions should be investigated. Extractions might be just as good and contamination less.

The Referee's attention has been called to the report of the Associate Referee on Zinc in Plants. This associate referee has also been troubled by more scatter in the results than desired. He dissolves his dithizone in carbon tetrachloride and makes a photometric mixed color dithizone determination with carbamate as a competitive complex. The Referee believes that since these two associate referees have many of the same difficulties, it might be well for them to collaborate and pursue parallel courses in the future.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the three recommendations made last year with respect to the determination of arsenic and antimony be repeated.

(2) That the study of micro methods for the determination of copper be continued, with special reference made to the determination of the lower limits of the carbamate method, the elimination of interferences, and the development of a possible all-dithizone method.

(3) That investigations on micro and spray residue quantities of fluorine be continued.

(4) That studies on micro and macro hydrocyanic acid determinations in foods be continued.

(5) That the changes in the official method for the determination of lead on apples suggested by the associate referee to accord with the recent increase in the tolerance be adopted, and that investigations on lead methods be continued.

(6) That investigation of mercury methods be continued.

(7) That the evaluation of selenium methods started by the associate referee be continued.

(8) That the investigation designed to improve the tentative method for the determination of micro quantities of zinc be continued, and that the efforts of the associate referees on the determination of zinc in plants, fertilizers, and foods be synchronized as far as possible.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 58 (1941).

## REPORT ON ARSENIC

By C. W. MURRAY (Eastern Regional Research Laboratory, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, Philadelphia, Pa.), *Associate Referee*

Since his appointment in the spring of the present year, the Associate Referee has been unable to do any chemical work on methods. Therefore, only the results of a collaborative study of the Cassil-Wichmann method, as modified by them, *This Journal*, 23, 297 (1940), will be presented at this time.

## RESULTS

The results obtained by the various collaborators are given in Table 1. The figures for the arsenic solutions are averages based on two or three determinations, and those for shrimp and tobacco are averages of from two to five determinations and two digestions.

TABLE 1.—*Collaborative results on three arsenic solutions and a sample each of shrimp and tobacco*

COLLABORATOR	ARSENIC SOLUTIONS			As <sub>2</sub> O <sub>3</sub> (P.P.M.)	
	NO. 1	NO. 2	NO. 3	SHRIMP	TOBACCO
	18.0	92.0	487.0		
American Can Co.					
O. F. Ecklund	17.2	96.4	465.0	5.0	35.6
California Dept. Agr.					
W. G. Marshall	16.9	89.3	480.0	3.1	37.1
Food and Drug Adm.					
S. Alfend	16.8	87.9	457.0	3.7	38.8
S. D. Fine	16.7	89.7	451.0	3.6	38.5
D. A. Ballard	16.5	88.7	464.2	4.6	33.7
L. W. Ferris	17.3	90.9	469.5	4.9	42.0
P. A. Mills	17.0	90.2	471.4	4.0	33.9 } 2d wet ash 39.8 } 3d wet ash 30.1 } 4th wet ash
A. K. Klein	17.6	88.9	477.0	4.5	33.3
D. M. Taylor	18.3	92.9	487.5	4.5	41.4
Bur. Ent. & Plant Quar.					
L. Koblitsky	19.5	99.6	449.0	7.1	40.4
J. E. Fahey	17.4	90.5	474.6	3.9	39.7
J. F. Cassidy	18.5	89.7	450.0	4.7	34.7
C. C. Cassil	19.6	92.3	480.0	6.6	39.6
J. H. Jones	18.7	88.4	453.0	—	—
Average	17.7	91.1	466.4	4.6	37.6
Av. % recovered	98.3	99.0	95.8	—	—
Range of recovery	91.7–	95.5–	92.2–		
Percentages	108.9	108.3	100.1	—	—

The comments of the various collaborators may be summarized as follows:

(1) The evolution of arsine is accompanied by too much foaming even after the addition of lead acetate.

(2) Several investigators had difficulty in dissolving the brownish-yellow deposit on the leucite tube.

(3) The solution can not be brought to boiling in 2 minutes because of the rapid evolution of hydrogen. The boiling point is reached only near the end of the evolution.

(4) In preliminary runs prior to actual analysis of the samples, several investigators report low recoveries—some as low as 92.5%.

(5) Several investigators are not satisfied with their results for Sample 3, and they suggest that this may be the limit of the method.

(6) Some doubt as to the accuracy of the shrimp analyses is raised.

(7) It is pointed out that by actual test all the arsine is not evolved in 7 minutes.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the text be revised to permit longer arsine evolution, perhaps 10 minutes; to bring the solution to a boil more slowly (5 or 6 minutes); and to provide that the precipitate on the leucite tube be given a minute or two to dissolve.

(2) That the upper limits claimed for the method be restudied as well as the analysis for arsenic in shrimp.

(3) That since the results of the tobacco analyses were only fair this study be repeated and that a peroxide combustion be tested as a possible replacement operation for the usual wet digestion now used for shrimp and tobacco.

#### REPORT ON COPPER

By C. A. GREENLEAF (National Cannery Association,  
Washington, D. C.), *Associate Referee*

The reports on copper during the past several years have established the merits of the colorimetric method with sodium diethyldithiocarbamate as a reagent and have demonstrated most of the necessary conditions for its successful use. The principal task this year was to consolidate this information in explicit form. In addition, further study was given to methods for overcoming the interference of bismuth, cobalt, and nickel.

In the latter phase of the work attention was given to the hydrogen sulfide separation and to a preliminary extraction with dithizone. The hydrogen sulfide separation was included in the collaborative work reported by Coulson, *This Journal*, 19, 219 (1936), who discussed the merits of this method for separation of small quantities of copper. Apparently

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 58 (1941).

the limitations of the sulfide method are imposed not so much by the solubility of copper sulfide as by the difficulty of inducing complete precipitation, which may take considerable time. Experience with the lead methods, in which copper is used as a gatherer for lead in the sulfide precipitation, suggested the possibility of accelerating the precipitation of copper sulfide by some such means. Accordingly some recovery experiments were made with various gatherers. The volume was 50 ml. in each case, pH adjusted to 2.0 (thymol blue). Each sample was sulfided and filtered immediately on a fritted filter, the subsequent determination being carried out according to the procedure later submitted to collaborators.

TABLE 1.—*Effect of gatherers on precipitation of Cu by H<sub>2</sub>S*

Cu TAKEN	Cu RECOVERED, MICROGRAMS			
	GATHERER—			
	PAPER PULP	Cd, 5 MG.	Pb, 5 MG.	Sn, 10 MG.
<i>micrograms</i>				
5	0.4	0.5	4.2, 3.5	3.2
10	9.1	5.7	11.3, 8.3	6.5
15			13.8	8.4
20			17.8	16.5
50	43.3	46.0	51.7	

Clearly, lead was the most promising of these gatherers, and further recovery experiments were carried out as shown in Table 2. While not consistent throughout, these results indicate a loss of copper somewhere between 0 and 0.5 microgram in a volume of 50 ml. This loss is probably serious only on quantities of 5 micrograms or less, but it indicates that the sulfide method is on safest ground when the quantity of copper is substantially greater than 5 micrograms. The loss would doubtless be less in a smaller volume, but it is not always possible to reduce the volume of solution to 10–20 ml. if the weight of sample is large.

TABLE 2.—*Effect of 5 mg. of Pb as gatherer on precipitation of Cu by H<sub>2</sub>S*

Cu TAKEN	TREATMENT BETWEEN SULFIDING AND FILTRATION	Cu RECOVERED
<i>micrograms</i>		<i>micrograms</i>
5	Shaken 30 minutes	4.57 4.54
5	Let stand overnight	5.41 4.85
5	Let stand 3 days	4.93 4.74 4.57 4.51

Dithizone extraction of copper followed by colorimetric estimation by the carbamate method was recommended by Sylvester and Lampitt.<sup>1</sup> Their procedure involves evaporation of the dithizone extracts to dryness and digestion with sulfuric and perchloric acids. Investigation showed that the copper could be recovered quantitatively from the dithizone extracts either by shaking with 6 *N* hydrochloric acid or by oxidizing the dithizone with bromine and shaking out with 1 *N* hydrochloric acid. The advantages of such a procedure would be recovery of the solvent and, it was hoped, elimination of the evaporation and digestion of the dithizone extracts. It was found, however, that oxidation products of dithizone gave false readings for copper in the subsequent carbamate determination. The error from this source was large in the case of the bromine procedure and small but variable in the procedure specifying 6 *N* hydrochloric acid. In the extraction with 6 *N* hydrochloric acid the error could be kept under control by the use of fresh dithizone reagent, but it was still a source of uncertainty that would be better eliminated. It was therefore decided to retain the digestion step but apply it to the aqueous solution obtained by shaking the dithizone extract with 1 *N* hydrochloric acid and bromine water. This permits recovery of the solvent and reduces the amount of organic matter to be destroyed by the sulfuric and perchloric acids.

Published data<sup>2</sup> on the dithizonates of copper, nickel, cobalt, and bismuth indicate that it should be possible to separate copper from nickel and cobalt by extraction at any *pH* below, say, *pH* 4 or 5, since their optimum extraction range is from *pH* 7 to 8. With bismuth the situation is not so clear. Although the optimum *pH* is said to be above 7, extraction may be made at much lower *pH* by using a sufficient excess of dithizone. Indeed Willoughby<sup>3</sup> separates bismuth from lead in this way by extracting at *pH* 2.

Recovery experiments with nickel and cobalt indicated that there was some slight extraction at *pH* 3–4. At this *pH*, when a solution containing 1 mg. of either nickel or cobalt was shaken with an amount of dithizone found suitable for 0.05 mg. of copper, enough metal was carried through the determination to simulate 6 micrograms of copper. At *pH* 2 interference by nickel or cobalt could not be detected.

The effect of *pH* and dithizone concentration on the extraction of copper and bismuth was partially explored, and the results are shown in Table 3.

In each instance the metal was contained in 25 ml. of aqueous solution, which was shaken with 25 ml. of a carbon tetrachloride solution of dithizone of the calculated composition. Copper was determined by a mixed color procedure in the dithizone solution, and bismuth by the carbamate

<sup>1</sup> *Analyst*, 60, 376 (1935).

<sup>2</sup> Fischer, Hellmut, *Angew. Chem.*, 50, 919–32 (1937).

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 7, 285 (1935).

method. The pH of the various solutions was determined with a glass electrode instrument.

The results in Table 3 are shown graphically in Figure 1. It is clear that there is too much overlapping of the copper and bismuth ranges to permit the ignoring of bismuth in the dithizone extraction. At the same time there is reason to think that a differential extraction may be made by using very dilute dithizone solutions and following the course of extraction by noting color changes. In this way the amount of excess dithizone could

TABLE 3.—*Extraction of Cu and Bi with dithizone in CCl<sub>4</sub> solution*

pH	NONE	EXCESS DITHIZONE	
		50%	650%
<i>Per cent extracted</i>			
<i>(a) Cu, 25 micrograms</i>			
-0.6	0	0	41
-0.4	11	16	85
0.2	60	72	100
1.2	89	100	100
2.2	100	100	—
3.2	100	100	—
<i>(b) Bi, 250 micrograms</i>			
		EXCESS DITHIZONE	
	NONE	100%	900%
0.2	0	0	0
1.2	12	48	100
2.2	48	87	100

probably be controlled to such a degree that most of the bismuth could be removed by shaking the extract with an acid solution of pH 1, without any serious loss of copper. However, this possibility was not developed in time to study it in a systematic way this year, although some of the collaborative results did encourage the belief that a suitable technic can be worked out.

The choice of a solvent for extraction of the carbamate complex is largely one of individual preference. Drabkin, *This Journal*, 22, 320 (1939), showed that isoamyl acetate is superior to amyl alcohol with respect to deviations from Beer's law and on the ground of lower volatility is superior to carbon tetrachloride. He also criticized the use of separatory funnels because of the risk of contamination with stopcock grease. However, this hazard can be overcome by the use of colorless petrolatum as stopcock lubricant, and many chemists prefer to use separatory funnels. Comparison was made of isoamyl acetate, carbon tetrachloride, and bromobenzene in calibrating a neutral wedge photometer, with the results shown in Table 4.

In each case there was some deviation from linearity, and the calibration curve could be represented considerably better by a second degree

TABLE 4.—Comparison of solvents for extraction of *Cu* diethyldithiocarbamate

Cu	SOLVENT					
	ISOAMYL ACETATE		CARBON TETRACHLORIDE		BROMOBENZENE	
	micrograms		PHOTOMETER READING			
	ACTUAL	NET	ACTUAL	NET	ACTUAL	NET
0	1.8	0.0	1.0	0.0	1.0	0.0
10	18.8	17.0	18.0	17.0	18.0	17.0
20	33.9	32.1	33.3	32.3	33.7	32.7
30	48.4	46.6	48.6	47.6	50.3	49.3
40	61.4	59.6	60.5	59.5	63.8	62.8
50	75.2	73.4	75.7	74.7	77.7	76.7

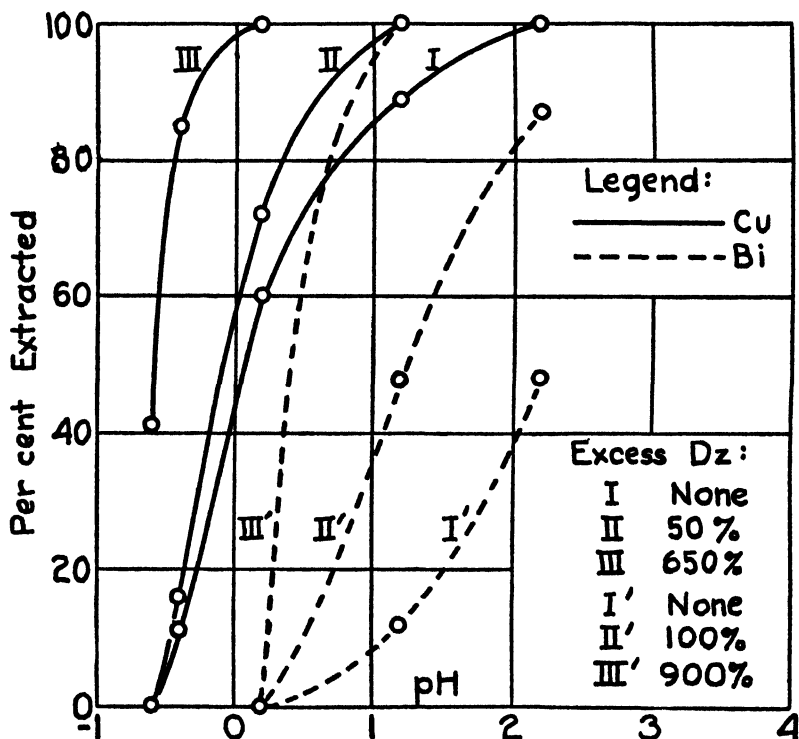
equation than by a straight line. Equations calculated from the net readings by the method of least squares were as follows:

Isoamyl acetate :  $\text{Cu} = 0.5765P + 0.001471P^2$ ;

Carbon tetrachloride:  $\text{Cu} = 0.5801P + 0.001268P^2$ ; and

Bromobenzene :  $\text{Cu} = 0.5630P + 0.001147P^2$ ,

where Cu = micrograms of copper in 10 ml. of solvent and P = photometer

FIG. 1.—EXTRACTION OF CU AND BI WITH DITHIZONE IN  $\text{CCl}_4$  SOLUTION.



reading from a 1 inch cell and No. 44 filter. The coefficient of the second degree term was smallest in the case of bromobenzene, indicating least deviation from Beer's law. Bromobenzene has a low vapor pressure at ordinary temperatures and thus can be handled without special precautions to prevent evaporation.

A single sample solution was submitted to collaborators for comparison of the hydrogen sulfide and dithizone separations. In both procedures the bismuth interference was eliminated by the use of cyanide in a duplicate determination as suggested by Drabkin, *ibid.* The sample solution was a synthetic one simulating spinach ash except for the absence of silica, and was based on the analyses reported by True, Black, and Kelly.<sup>4</sup> The constituents were freed from copper so far as possible by hydrogen sulfide, and the finished solution, before addition of copper, bismuth, cobalt, and nickel, was analyzed for copper by the carbamate method. The quantity found corresponded to 0.18 mg. per liter of finished solution, and 2 mg. per liter was added. The sample solution submitted to collaborators represented, per liter:

	Grams
"Dried spinach" (20% ash) . . . . .	400
	Mg.
Nickel . . . . .	50
Cobalt . . . . .	50
Zinc . . . . .	100
Tin . . . . .	100
Bismuth . . . . .	15
Copper (added) . . . . .	2
Copper (total) . . . . .	2.18

The following instructions were furnished to collaborators:

#### DETERMINATION OF COPPER, A.O.A.C.—1940

The work requested consists of examination of a single sample solution by two procedures designed to determine copper in the presence of cobalt, nickel, and bismuth, metals that interfere in the carbamate method.

A photometer of some sort is needed, either a neutral wedge photometer or a photoelectric instrument. It is assumed that the instrument can be calibrated either in the range 0–50 or 0–20 micrograms of copper, depending on the type of instrument.

Results at least in duplicate by each of the two methods are requested. With blanks this involves at least six determinations or twelve final photometer readings. Collaborators are asked to comment freely.

#### REAGENTS AND APPARATUS

Water redistilled from Pyrex glass for all dilutions. All reagents C.P.

Separatory funnels, 125 ml. capacity, marked at 55 ml. Grease lower stopcocks with white petrolatum and do not use brass chains.

Rinse glassware with HNO<sub>3</sub> and finally with distilled water.

<sup>4</sup> *J. Agr. Research*, 16, 15 (1919).

(a) *Hydrochloric acid*.—6 *N*. Dilute concentrated acid (1+1) with water and redistil in Pyrex glass.

(b) *Hydrochloric acid*.—*N*/1. Dilute 6 *N* acid (1+5).

(c) *Nitric acid*.—C.P. Preferably redistilled from Pyrex glass.

(d) *Dithizone solution*.—100 mg./liter in  $\text{CCl}_4$ .

(e) *Lead nitrate solution*.—1.6 grams/liter.

(f) *Wash solution for sulfides*.—30 grams of  $\text{Na}_2\text{SO}_4$  plus 20 ml. of 6 *N* HCl(a) per liter, saturated with  $\text{H}_2\text{S}$  just before use.

(g) *Ammonium citrate reagent*.—Dissolve 150 grams of citric acid in water and make just alkaline to litmus with  $\text{NH}_4\text{OH}$  (180–190 ml.). Add 10 ml. of formaldehyde and 5 ml. of the carbamate reagent and make up to 1 liter. Purify by extracting several times with small portions of  $\text{CCl}_4$ , shaking the solution vigorously at each extraction. Finally filter to remove any suspended  $\text{CCl}_4$ .

(h) *Sodium diethyldithiocarbamate solution*.—Dissolve 1 gram of the salt in water, make up to 100 ml., and filter.

(i) *Copper standard*.—Weigh accurately 0.2000 gram of C.P. Cu wire or foil and place in a 125 ml. Erlenmeyer flask. Add 15 ml. of  $\text{HNO}_3$ (1+4), cover with a watch-glass, and allow to dissolve, warming to complete solution. Boil to expel fumes, then cool and dilute to 200 ml. Prepare an intermediate standard by diluting 20 ml. of the first solution to 200 ml. This intermediate standard contains 0.1 mg. per ml. The working standard, prepared just before use, consists of either 20 ml. or 10 ml. of the intermediate standard made up to 1 liter, and contains 2 or 1 microgram of Cu per ml. Use the former if the range 0–50 micrograms is to be covered, or the latter if 0–20.

#### PRELIMINARY SEPARATION

Run blanks throughout by both methods.

##### *I. Hydrogen Sulfide Method*

To 25 ml. of the sample solution in a 125 ml. Erlenmeyer flask add 10 ml. of the  $\text{NH}_4$  citrate solution, 5 ml. of the  $\text{Pb}(\text{NO}_3)_2$  solution (Note 1), and 3 drops of 0.04% solution of thymol blue. Adjust the pH to approximately 2.0 (faint pink color of indicator) by dropwise addition of the 6 *N* HCl and pass in a rapid stream of  $\text{H}_2\text{S}$  for 2–3 minutes. The  $\text{H}_2\text{S}$  should be washed in a gas washing bottle containing water.

Stopper the flask tightly and let stand overnight or place on a shaking machine for 1 hour. Filter with suction on a fine fritted filter (Jena 11G4 or equivalent), washing the flask and precipitate with a few small portions of the wash solution. Transfer filter and flask to a bell jar and dissolve the mixed sulfides by adding 2 ml. of the 6 *N* HCl followed by 3 ml. of hot  $\text{HNO}_3$ . Filter with suction and wash the filter with water, drawing the filtrate and washings into the original flask. Evaporate to a volume of 15–20 ml., cool, and transfer to a 50 ml. volumetric flask. Make up to volume and examine for Cu as directed under "Estimation of Copper."

##### *II. Dithizone Extraction*

To 25 ml. of the sample solution in a separatory funnel add 10 ml. of the  $\text{NH}_4$  citrate solution and 3 drops of the thymol blue. Adjust the pH to approximately 2.0 (faint pink color of indicator) by dropwise addition of the 6 *N* HCl. Extract with successive 10 ml. portions of the dithizone solution, shaking vigorously for several minutes, until no change is produced in the green color of the dithizone (Note 2). Discard the aqueous layer. Combine the extracts in a second separatory funnel and wash once with 10 ml. of 0.1 *N* HCl. Draw off the  $\text{CCl}_4$  layer into a clean separatory funnel, wash the HCl with 5 ml. of  $\text{CCl}_4$ , add this washing to the dithizone solution, and discard the HCl. Add 10 ml. of 1 *N* HCl and 5 drops of Br water

(Note 3). Shake thoroughly, allow to separate, draw off, and discard the  $\text{CCl}_4$  layer. Draw off the aqueous layer into a 100 ml. beaker and wash the separatory funnel with several small portions of water, disregarding any droplets of  $\text{CCl}_4$  that may be included. Add 1 ml. of concentrated  $\text{H}_2\text{SO}_4$  and evaporate on a hot plate to incipient white fumes. Add 1 ml. of  $\text{HNO}_3$  and 1 ml. of 60-70%  $\text{HClO}_4$  and heat again until all or most of the  $\text{HClO}_4$  is expelled, as indicated by the residual volume (Note 4). Cool, wash into a 50 ml. volumetric flask, and make up to volume. Examine for Cu as directed under "Estimation of Copper."

#### ESTIMATION OF COPPER

If the photometer is calibrated in the range 0-50 micrograms pipet 20 ml. of the solution obtained as directed above to each of two separatory funnels. If the range 0-20 micrograms is covered, use 10 ml. Add 10 ml. of the  $\text{NH}_4$  citrate reagent and 3 drops of 0.02% solution of cresol red to each and add  $\text{NH}_4\text{OH}$  dropwise to a distinct purplish red color. Designate the two aliquots as *A* and *B*, and to *B* add 5 ml. of 10% KCN solution. Add 1 ml. of the carbamate solution and dilute to 55 ml. Add exactly 10 ml. of  $\text{CCl}_4$  or bromobenzene (Note 6). Shake vigorously for 2 minutes and allow the layers to separate. Draw off the lower layer through a pledget of cotton in a funnel into a suitable container (Note 6) and determine the absorption, transmission, or scale reading in a suitable photometer or photoelectric colorimeter, utilizing monochromatic or nearly monochromatic light at a central wavelength of approximately 440 millimicrons. From the reading obtained compute the apparent amount of Cu in each determination and blank according to the calibration of the instrument.

#### CALIBRATION OF PHOTOMETER

1. *Neutral wedge photometer.*—Prepare a series of standards containing 0-50 or 0-20 micrograms of Cu by transferring suitable volumes of the dilute copper standard to separatory funnels and proceeding as directed under "Estimation of Copper," beginning "Add 10 ml. of the  $\text{NH}_4$  citrate reagent," but of course omitting KCN. From the readings determine the slope of the calibration curve either graphically or by calculation according to the scheme given under "Photometric Methods," *Methods of Analysis, A.O.A.C.*, 1935, 385. Adjust the photometer to read zero with the pure solvent and use the slope of the calibration curve as a factor to convert readings to copper.

The range 0-50 micrograms will usually give convenient readings if a 1-inch absorption cell is used and the 0-20 range with a 2-inch cell.

2. *Photoelectric instruments reading in per cent transmission.*—The only difference from the foregoing instructions is due to the relationship between absorption and transmission. Calibration curves can be plotted on semilog paper, per cent transmission being plotted on the logarithmic scale and copper on the linear scale. Due to the extraction blank the line will generally not pass through the point representing 100% transmission for the solvent, but the true calibration line may be drawn through this point parallel to the line obtained from the standards.

#### CALCULATION OF RESULTS

Having all determinations and blanks converted to apparent amounts of Cu, subtract from each determination (*A* and *B*) its corresponding blank. The difference between the net values for *A* and *B* represents the Cu in the aliquot extracted. If this was 20 ml., divide micrograms of Cu by 10 to give mg. per liter in the original sample solution, or if a 10 ml. aliquot was taken divide by 5. Report in mg. per liter.

## NOTES

1. The Pb is added as a gatherer. It is extracted with Cu in the final stage but the Pb compound is colorless.

2. Extraction with dithizone from acid solutions proceeds slowly and requires protracted shaking.

3. Bromine does not completely decolorize dithizone but produces a yellow color in the  $\text{CCl}_4$  layer.

4. Complete oxidation is essential at this point to avoid later interference due to intermediate oxidation products of dithizone.

5. Cyanide completely prevents extraction of Cu due to complex formation. No complex is formed with Bi.

6. If the final measurement can be completed without undue risk of evaporation of solvent,  $\text{CCl}_4$  is suitable. If this is used, it should be drawn into small stoppered flasks or bottles pending final examination. Bromobenzene, owing to its low vapor tension, can be handled in open test tubes and absorption cells without appreciable concentration due to evaporation.

Collaborative results are given in Table 5.

TABLE 5.—*Collaborative results on determination of Cu*

COLLABORATOR	$\text{H}_2\text{S}$ METHOD	Cu
		DITHIZONE METHOD
		<i>mg./liter</i>
Andrew G. Buell, Food & Drug Adm., San Francisco	2.08	1.91
	1.93	1.81
Herzl Cohen, Food & Drug Adm., Chicago	1.6*	1.5*
	1.5*	1.4*
E. Ferris, Food & Drug Adm., Buffalo	2.03	2.03
	2.02	2.00
Norman E. Foster, Food & Drug Adm., Philadelphia	2.32	1.94
	2.18	1.82
A. K. Klein, Food & Drug Adm., Washington	2.01	2.11
	1.99	2.04
		2.04
Frank C. Lamb, National Cannery Assoc., San Francisco	2.40	2.14
	2.38	2.14
	2.30	2.12
E. D. Sallee & O. R. Alexander, American Can Company, Maywood	2.1	1.9
	2.1	1.9
C. A. Greenleaf	1.87	1.94
	1.86	1.93
	1.85	1.93
	1.83	1.92
G. H. Bendix, Continental Can Co., Chicago	2.18*	Cloudy extract
	2.30*	Cloudy extract
	1.51*	4.80*
	1.10*	5.30*
Average	2.07	1.98

\* Not included in average.

## COMMENTS BY COLLABORATORS

*Andrew G. Buell.*—It was noted in the hydrogen sulfide method that when the precipitated sulfides were permitted to stand overnight a white precipitate was formed. Therefore these determinations were discarded, and the alternative procedure of precipitating the sulfides and shaking for 1 hour was used. No white precipitate was formed when this procedure was used.

*Herzl Cohen.*—When solutions of known Cu concentration were taken, only about 70% of the Cu was recovered. In spite of the number of determinations made, I am unable to suggest at what point or points in the procedure the losses of Cu occur.

TAKEN (MG./LITER)	FOUND (MG./LITER)			
	H <sub>2</sub> S		DITHIZONE	
2.0	1.1,	1.1	1.5, 1.5,	1.6 1.5
1.0	0.8		0.7,	0.7

*E. Ferris.*—Estimation of Cu extracted was measured on a photometer made in the Food and Drug Administration, Washington, D. C., by using light filter No. 46 and standard range 0–50 gamma of Cu. The calibration curve was a straight line from 0 to 30 gamma. Above this there was a slight dropping of the photometric reading.

*Norman E. Foster.*—No difficulties were encountered in the method, and the directions were adequate. The photometer was standardized in the 0–20 range and bromobenzene was used as the solvent.

Three filters were used in the photometer, Nos. 44, 45, and 46. With Filter No. 44, the two halves of the field were of different tints and matching was difficult, especially in the higher concentrations. Filter No. 46 gave the nearest matching tints and was the easiest to use. The curves for each filter showed no apparent departure from linearity.

*A. K. Klein.*—The No. 44 filter is not quite an exact match for the color of the 30 gamma extraction standard. The developed color has slightly more red in it.

Of the two procedures, I much prefer the one specifying dithizone extraction to the one requiring H<sub>2</sub>S precipitation. The first is handier and the color read is due solely to the Cu present. The contaminants, Co, Ni, and Bi were successfully eliminated before the final reading as evidenced by the cyanide blank. The H<sub>2</sub>S variation, however, is longer and, in addition, requires a micro filtration, something that most chemists like to avoid.

*Frank C. Lamb.*—We have obtained exact linearity upon plotting our calibration curve in the range 0–50 micrograms of Cu, using bromobenzene as extracting medium. The instrument used was a Cenco-Sheard-Sanford photometer equipped with a blue filter (Cenco No. 1).

Neither procedure offered any apparent difficulties in manipulation; however, it might be advantageous to use a weaker dithizone solution for the final extractions of Cu since slight changes of color are difficult to observe in too concentrated a solution.

*E. D. Sallee and O. R. Alexander.*—We are enclosing a copy of our calibration curve showing the relationship between micrograms of Cu and per cent transmission at a wave length of 434 millimicrons on our photoelectric colorimeter. Considerable

difficulty was experienced in adjusting the  $pH$  to 2.0 with thymol blue prior to the dithizone and  $H_2S$  separation.

In the dithizone extraction it was difficult to discern any change in the dithizone color owing to the fact that the reagent was so concentrated. Five extractions were made. There was a marked departure from linearity in the calibration curve, which necessitated several standards being run. The amount of Bi that came through five dithizone extractions was 0.33 mg. per liter in terms of apparent Cu.

*G. H. Bendix.*—We followed your directions exactly and for a colorimetric comparison we used a Coleman spectrophotometer. Determinations No. 1 and 2 were made with a 0–50 microgram calibration range and a 20 ml. aliquot. Determinations 3 and 4 were made with a 0–20 microgram calibration range and a 10 ml. aliquot as described in the procedure. In Determinations 1 and 2, the extract from the sulfide procedure became turbid before absorption readings were obtained. When the procedure in Determinations 3 and 4 was repeated the readings were taken as soon as the color developed and before they became turbid.

Our results are certainly not consistent, but we consider the dithizone method the better of the two. We like especially the theory of its operation and the speed with which it can be performed. With the sulfide method we find the turbidity of the extracts extremely objectionable. There is also some doubt in our minds as to whether Co and Ni can be separated completely by sulfiding at a  $pH$  of 2. We have no evidence available to show this, but we would suspect an appreciable absorption of Co and Ni on the voluminous Cu, Pb, and Bi sulfides.

#### DISCUSSIONS OF RESULTS

As it is evident from the comments made by Herzl Cohen that he was contending with some condition not encountered by the other collaborators and probably not inherent in the procedure, his results were not included in the average.

Examination of supplementary data submitted by the collaborators shows considerable variation in the quantity of bismuth extracted in the dithizone method. The Associate Referee made two extractions and found no appreciable quantity of bismuth at the end. A. K. Klein made three extractions and obtained similar results, as will be noted from his comments. However, E. D. Sallee and O. R. Alexander, who extracted with five portions of dithizone, found bismuth equivalent to 0.33 mg./liter of copper. Evidently if bismuth interference is to be eliminated by the dithizone extraction, it will be necessary to refine the technic of this step to permit differential extraction. While the dithizone method made practically as good a showing in the collaborative work as did the hydrogen sulfide method, and may be preferable with respect to manipulation, it is believed that further study will make it possible to eliminate the cyanide step, and consideration of the method for adoption will be more appropriate when that point has been clarified. In the meantime the hydrogen sulfide-cyanide procedure would furnish a workable micro method for copper in the presence of interfering metals. In the absence of bismuth, cobalt, and nickel the carbamate method may be applied directly to a solution obtained by wet combustion or ashing of the sample.

## RECOMMENDATIONS\*

The Associate Referee would perhaps be as fully justified in recommending the dithizone separation for adoption as the hydrogen sulfide procedure. However, it is believed that the dithizone separation can be further improved to eliminate bismuth without the use of cyanide and that it would be an economy of the Association's time to defer consideration until that possibility has been investigated. In the meantime it may develop that an all-dithizone method can be perfected; at present the extent of interference by other metals is not well enough charted to judge that possibility. Therefore it is recommended that further study be given to the dithizone separation, both with relation to the carbamate method and the possibility of an all-dithizone method.

## REPORT ON ZINC†

By E. B. HOLLAND and W. S. RITCHIE (Massachusetts  
Agricultural Experiment Station, Amherst, Mass.),  
*Associate Referee*

At the 1939 meeting the Association recommended that the method proposed by the Associate Referee on Zinc in Foodstuffs be adopted as tentative and that work be continued. In conformity thereto samples of white dent corn (Johnson County Ensilage) and of spinach (Burpee's Victoria), together with granulated zinc for standardizing, were prepared and sent to the chemists that had expressed a willingness to collaborate.

The analysts were cautioned to use only two grams of spinach (Sample 2) for the determination and to exercise extreme care in subsampling as the quantity of zinc was relatively high and the ash content about 20 per cent.

## COMMENTS

As most of the chemists collaborating had had no previous experience with the method their comments should be given careful consideration.

*H. F. Bergman and W. E. Truran.*—We experienced great difficulty from contamination by the glassware and even under the best control obtainable secured a high blank.

*W. Y. Gary.*—I employed a Fisher electrophotometer and a green Wratten filter and found it necessary to dilute the final  $\text{CCl}_4$  extract 1 to 5. Such solutions seemed to be more stable but must contain a considerable excess of dithizone to insure uniform results.

*Alfred K. Klein.*—The procedure worked easily but the greatest hazard was the blank control even when using mostly Pyrex. One solution for example increased from 2.4 to 10.4 gamma of zinc in two weeks. The zinc dithizone complex in  $\text{CCl}_4$  was found to be a much richer red than that of lead of the same concentration.

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 58 (1941).

† Contribution No. 386, Massachusetts Agricultural Experiment Station.

When zinc was extracted from solutions containing from 0 to 50 gamma with 40 ml. of 20 mg. of dithizone per liter of  $\text{CCl}_4$  the scale readings by a neutral wedge photometer versus gamma zinc concentration were almost a straight line function.

*J. W. Kuzmeski.*—I had no difficulty in the manipulation but the blanks were appreciable.

*J. H. Loughrey.*—The method seems to work very well except for the tendency to extract zinc from all glassware, even Pyrex. Paraffine coatings kept the blank between 1 and 2.5 gamma. I used solutions of dithizone in  $\text{CCl}_4$  of known strength to extract the zinc and found it preferable to  $\text{CHCl}_3$ . I employed a neutral wedge photometer with a green filter centered at 510 m $\mu$ .

*Harold B. Pierce.*—The reagents dissolved zinc even from Pyrex bottles and extractions were made before each day's analyses.

The results are summarized in Table 1.

TABLE 1.—*Summarized results*

	SAMPLE 1	SAMPLE 2
H. F. Bergman and W. E. Truran	27.47	131.85
Vernon Coutu	26.68	114.46
W. Y. Gary	29.00	110.00
Alfred K. Klein	31.67	105.28
J. W. Kuzmeski	32.00	128.86
J. H. Loughrey	25.50	120.00
M. H. Niccoli	22.68	43.30*
Harold B. Pierce	51.10*	87.62
Bernard A. Starrs	32.56	144.81
Average	28.45	117.86

\* Excluded from average.

The results are reasonably consistent for Sample 1 (corn of a low ash content) when the experience of the analysts is considered as well as the difficulty in controlling the blank, which varied from 0.65 to 6.88 p.p.m.

With Sample 2 (spinach of about 20 per cent ash) the results are not so satisfactory, possibly due in some cases to insufficient hydrochloric acid or boiling to completely dissolve the zinc in the ash.

An attempt was made to confirm the results by a spectrographic analysis of the ash, but the zinc content was too high in both samples to permit an accurate determination.

#### GENERAL OBSERVATIONS

The contamination of the solutions by zinc from the glassware is probably the most serious obstacle in the accurate determination of zinc in foodstuffs. Various solvents have been used to remove zinc from the glassware but these appear to have only a temporary effect. Protective coatings of wax proved serviceable for only a short time. Various plastics are being tested in hopes of securing a more resistant coating. Possibly the new high silica glass may meet the requirements.



Ashing at visible redness (500° C.) was not criticized by any of the analysts.

Incomplete solution of the zinc in the ash (noted in some instances) may have been due to haste.

Methyl red is preferable to litmus paper for the indicator.

The Duboscq comparator is subject to personal errors but permits the reading of much darker solutions than do the electrophotometers.

It is recommended\* that the work on the determination of zinc in food-stuffs be continued with special attention to insure:

- (a) Complete solution of the zinc in the hydrochloric acid.
- (b) Prevention or at least reduction of contamination by the glassware.
- (c) Use of dithizone in carbon tetrachloride when a suitable colorimeter and filter are available for a "mixed color" method.
- (d) Adoption of a specific color filter for the determination.

## REPORT ON FLUORINE

By P. A. CLIFFORD (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Several investigators<sup>1</sup> have spoken of a distillation blank or "positive perchlorate error" when pure perchloric acid is carried through a Willard and Winter<sup>2</sup> fluorine distillation. This effect was mentioned in the Associate Referee's report last year, *This Journal*, **23**, 303 (1940), and it was soon found that precise results on quantities of fluorine below 10 micrograms could not be obtained unless this blank was evaluated and controlled. It was noted that the blank increased with increased temperature of distillation and with increased acidity of the distillate, and these two closely allied effects pointed towards decomposition of perchloric acid as a cause.

It is known that perchloric acid has a slight tendency to decompose into chlorine and oxygen at temperatures above 120° C. (The bleaching effect of chlorine upon the zirconium-alizarin indicator has been discussed by Lockwood,<sup>3</sup> who used sodium nitrite to discharge the chlorine.) The tendency of perchloric acid to decompose would be increased by the superheating of the walls of the distilling flask, as when the latter is heated in the usual manner by a direct flame. Dahle, *This Journal*, **21**, 473 (1938), has shown how adequate shielding of the flask reduces the acidity of the distillate. The Associate Referee has found further that the use of a Wood's metal bath, in conjunction with the usual 8×8 inch transite board, will reduce acidities of perchloric acid distillates to almost negligible pro-

\* For report of Subcommittee C and action by the Association, see *This Journal*, **24**, 59 (1941).

<sup>1</sup> D. Dahle, *This Journal*, **21**, 208 (1938); **22**, 338 (1939); Ebers, Lamb, and Lachele, *J. Ind. Eng. Chem., Anal. Ed.*, **10**, 259 (1938); Reynolds and Hill, *Ibid.*, **11**, 21 (1939).

<sup>2</sup> *Ind. Eng. Chem., Anal. Ed.*, **5**, 7 (1933).

<sup>3</sup> *Analyst*, **62**, 775 (1937).

portions. The molten Wood's metal, contained in a large (4 inch) iron crucible, is maintained by a burner flame at 180°–200° C., and the distilling flask is immersed to a point where the level of the metal is somewhat below that of the boiling liquid in the flask. There is thus little chance of the side walls of the flask being superheated by burner gases or overheated air above the upper surface of the transite board, as when the latter alone is used for shielding. On the theory that part of the blank, at least, is due to the bleaching effect of chlorine upon the indicator, the practice of adding 1.0 ml. of a 0.1 per cent solution of hydroxylamine hydrochloride to both the "sample" and "blank" tubes was adopted when the back-titration method was applied to distillates (Method A, vide infra). This amount was adequate to discharge the traces of chlorine sometimes encountered.

As a further precaution there was adopted the use of all-glass apparatus, consisting of several units with interchangeable condensers, thermometers, and dropping funnels. Eberz et al.<sup>1</sup> state that the use of all-glass apparatus lowers the distillation blank, and this has been the general, but not invariable, experience of the Associate Referee. Two interchangeable sizes of distilling flasks were supplied per unit, one of about 100 ml. capacity and a larger one of 250 ml. size for use in special cases, as when organic samples are distilled from sulfuric acid. Water redistilled from alkaline permanganate has been used exclusively.

These refinements resulted in the lowering of the blank to such a point that it represented less than 1.0 microgram of fluorine per 150 ml. of distillate, and could not be evaluated precisely by a single determination. Accordingly, 10 blank distillations were made from the several stills, 150 ml. of distillate being collected at 135° C. each time. The 60 per cent perchloric acid used had been diluted with 3–4 volumes of water and boiled down to original strength (20 ml. per distillation). The stills were given a routine washing with hot 10 per cent sodium hydroxide between runs in order to remove traces of gelatinous silica, Reynolds, D. S., *This Journal*, 18, 108 (1935), and Reynolds and Hill.<sup>1</sup> The distillates were treated with a slight excess of 1 per cent sodium carbonate solution (F free) prepared from the twice-recrystallized salt, and evaporated successively to small volume in the same platinum dish. The solution was then transferred back to the still, redistilled from a further portion of perchloric acid, and the fluorine was determined in the next 150 ml. of distillate. Results by Method A, checked by Method B (vide infra) gave a figure of 9.8 micrograms, or 0.9 microgram of fluorine per distillation. An identical figure was obtained when the distillation was continuous, 10 consecutive 150 ml. fractions being collected from the same still and same batch of acid, but a fresh 20 ml. of perchloric acid being used for the final distillation.

These results indicate that the small blank noted per distillation is actually fluorine and that this fluorine does not come from the perchloric

acid. Presumably it is leached from the glass of the still and volatilized during the distillation. A sample of Pyrex beaker glass, analyzed by Willard and Winter's<sup>2</sup> technic, gave a result for fluorine of 0.02 per cent. Thus, to produce 1.0 microgram of fluorine 5 mg. of glass would be decomposed per distillation. This does not seem to be an unreasonable figure. The Associate Referee sees no simple way of eliminating this small blank.

The figures in Table 1 are presented to illustrate the necessity of taking the distillation blank into account when small samples of comparatively low fluorine content are analyzed. The material was raw veal bone ground to 40-mesh size. Samples of various weights were introduced into the distilling flasks and distilled directly out of 20 ml. of perchloric acid. The distillates (150 ml.) were treated with an excess of fluorine-free lime water, dried in platinum on the steam bath, then ashed at 550° C. for 1 hour. The ashed residues were transferred to the still, and the distillation was repeated. Sample weights, total fluorine recovered, and fluorine content in p.p.m., corrected for the distillation blanks (2×0.9 micrograms) are listed in Table 1. The last column lists the results that would have been obtained if distillation blanks had not been subtracted.

TABLE 1.—*Results showing necessity of allowing for distillation blanks when small samples of low F content are analyzed*

WT. SAMPLE	TOTAL F	TOTAL F—BLANK	F	F
grams	micrograms	micrograms	(p.p.m.)	(p.p.m.)
.25	3.6	1.8	7.2	14.4
.50	5.6	3.8	7.6	11.2
1.00	8.6	6.8	6.8	8.6
2.00	16.8	15.0	7.5	8.4
5.00	37.0	35.2	7.0	7.4

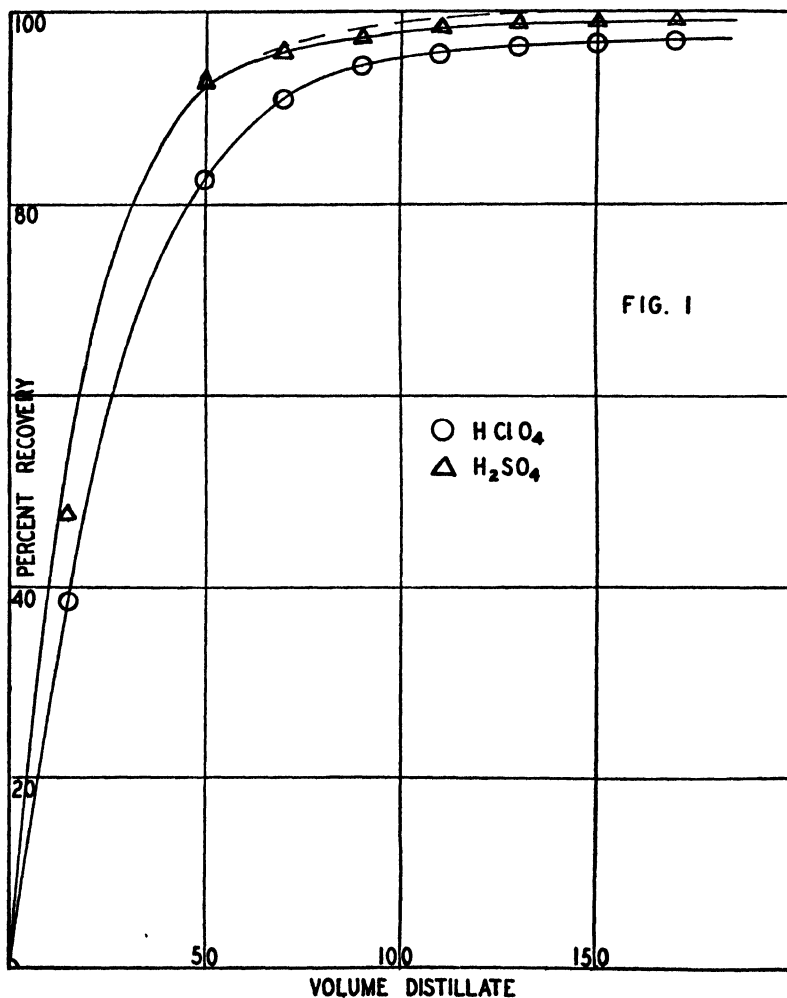
The distillation blank with sulfuric acid was evaluated in a similar manner, first with 10 separate distillations with 20 ml. portions of 1+1 acid at 135° C. and then with 10 consecutive distillations from the same acid. Previously the sulfuric acid had been diluted with 4–5 volumes of water and boiled down to about 1+1 strength. This should remove fluorine and also the volatile impurity (probably sulfur dioxide) mentioned by Hoskins and Ferris.<sup>4</sup> In each case the final distillation was from perchloric acid. When distillations were made separately the average blank was 0.8 microgram of fluorine, and when made consecutively the average amount of fluorine per 150 ml. of distillate was 0.4 microgram.

#### RETENTION IN THE DISTILLATION

The positive distillation blank may mask a tendency towards incomplete volatilization of fluorine. Eberz et al.<sup>1</sup> state that "results depend

<sup>4</sup> *Ind. Eng. Chem., Anal. Ed.*, 8, 6 (1936).

upon the compensation of incomplete distillation by the positive perchlorate error." Dahle, *This Journal*, 22, 338 (1939), makes a similar observation. The Associate Referee's experience is that a 100 per cent recovery is practically never obtained when quantities of fluorine up to 200 micrograms are distilled from perchloric acid, and 150 ml. of distillate is collected at 135° C. Errors of the methods used would hardly account for these losses. In the following studies results were checked wherever possible by both the methods later described. The case is somewhat better with sulfuric acid, but here also a slight repression is noted. Typical recovery curves are given in Figure 1, where with 200 micrograms of fluorine



in each case, the indicated volumes of distillate were analyzed separately, and the cumulative amounts of fluorine were plotted as per cent recovery against distillate volume.

Dahle and Wichmann, *Ibid.*, 19, 313 (1936), used the equation,  $k = 1/v \log c/c-x$ , where  $k$ =constant,  $v$ =volume of distillate,  $c$ =original concentration of fluorine in the flask, and  $c-x$ =the concentration after  $v$  ml. of distillate has been collected, to evaluate the relation between volume of distillate and percentage recovery. The value of  $k$  depends upon several factors: temperature of distillation, volume of acid used, and the individual flask. These authors distilled from sulfuric acid. They point out how mathematical treatment of this relationship would enable the analyst to calculate the total amount of fluorine being distilled by analyzing the first 25 or even 10 ml. of distillate, thus rendering unnecessary a complete distillation. Koehler<sup>4</sup> uses a similar expression in the analysis of lime under conditions showing slow evolution of fluorine from perchloric acid. However, in the Associate Referee's experience the direct logarithmic relationship does not hold for perchloric acid, as Koehler notes when dealing with small quantities of fluorine. The calculated values of  $k$  rapidly diminish when observed values of  $x$  for increasing values of  $v$  are substituted in the formula. The equation,  $k = 1/v - 0.0033 v^2 \log c/c-x$ , fits the experimental data well, as it indicates the best possible recovery as 96 per cent with 150 ml. of distillate. (The arbitrary correction factor  $0.0033 v^2$  was chosen because it gives fairly constant values of  $k$ . The quadratic resulting from its use is indefinite for recoveries above 96 per cent, and except at this point when solved for  $v$  gives two roots, of which the smaller should, of course, be chosen.)

The data for sulfuric acid plotted in Figure 1 likewise indicates a slight departure from the logarithmic relationship between volume and percentage recovery, and here again the Associate Referee checks the observations of Eberz et al. However, sulfuric acid seems to be the most effective distilling acid.

The collection of larger volumes of distillate does not solve the difficulty. Thus, when 200 micrograms of fluorine was distilled out of perchloric acid the first 150 ml. of distillate (at 135° C.) contained 193 micrograms of fluorine. A second 150 ml. fraction (corrected for the fluorine blank) indicated no fluorine, and a third fraction likewise indicated none. Without cleaning the still, and with the same acid, the analyst introduced another 200 micrograms of fluorine and repeated the experiment. Only 186 micrograms of fluorine appeared in the first distillate, and the second and third again indicated negligible quantities. When the experiment was repeated a third time, 187 micrograms of fluorine appeared in the first 150 ml. fraction, while 2.0 and 0.7 micrograms appeared in the second and third, respectively. In a fourth experiment, strangely enough, a complete recovery was noted in the first 150 ml. fraction, and the second and third fractions yielded quantities below 1 microgram. A complete recovery was noted in the first 150 ml. fraction when the experiment was repeated a

<sup>4</sup> Symposium on Lime, A.S.T.M. Columbus Regional Meeting, March 8, 1939.

fifth time. The reason for this shift to good recoveries is not known. After five distillations a total of about 30 micrograms out of 1000 was still unrecovered, and on the theory that a higher distillation temperature might release this retained fluorine, a further 100 ml. of distillate was collected from this same acid at fuming temperature ( $155^{\circ}$ – $165^{\circ}$  C.). The distillate, which contained considerable acid, was neutralized with a few ml. of fluorine-free lime water, evaporated down, and redistilled out of 20 ml. fresh perchloric acid. Only 4.5 micrograms of fluorine was recovered.

The cause of this retention of fluorine remains a matter of conjecture. The Associate Referee has thought that treatment of the still with hot sodium hydroxide might leave its interior surface in a hydrated, "gelatinous" condition with the conceivable possibility that traces of fluorine might be adsorbed possibly as a non-volatile oxyfluoride.<sup>6</sup> It was found that when the stills were fumed out with boiling sulfuric acid and rinsed thoroughly with water immediately before use recoveries with perchloric acid rose to 98.4 per cent on quantities of fluorine up to 200 micrograms (av. of 12). The distillation blank was rechecked as before by a series of 10 distillations and was found not to be increased by this fuming procedure. The effectiveness of this treatment was further checked as follows: With an original 200 micrograms of fluorine, five consecutive distillations were made from perchloric acid, and the distillates were evaporated down in platinum each time after being neutralized with fluorine-free alkali, and redistilled from fresh acid. In the first case the still was given the routine, rinse with hot alkali between runs, and the sulfuric acid fuming was omitted. Analysis of the fifth distillate yielded 179 micrograms of fluorine, a recovery of 89.5 per cent. When the still was given an additional fuming with boiling sulfuric acid between runs, a similar experiment yielded upon the fifth distillation a recovery of 93 per cent. The higher recovery in the latter case would indicate that "dehydrating" the still with a sulfuric acid fuming, or by other means, is a desirable procedure.

A few experiments were also made to check recoveries with sulfuric acid at elevated temperatures. Thus 200 micrograms of fluorine in 50 ml. of water, plus 20 ml. of 1+1 sulfuric acid, was introduced into the flask and distilled rapidly to the appearance of fumes (approximately  $170^{\circ}$  C.). No water was introduced during the distillation. The distillate, about 60 ml., was made to 100 and analyzed by Method B. Only 75 per cent of the total fluorine was distilled. When the experiment was repeated and the still was held at fuming temperature for 10 minutes, 87.5 per cent was recovered. The acidity of the distillate was equal to 6.4 ml. of 0.1 *N* alkali. The presence of water seems essential, and recovery of fluorine as  $\text{H}_2\text{SiF}_6$  is of the nature of a steam distillation. When 200 micrograms of fluorine was distilled as usual out of 20 ml. of 1+1 sulfuric acid at  $145^{\circ}$  C., 98.5 per cent appeared in 100 ml. of distillate. Traces of acid appeared,

<sup>6</sup> Reynolds and Jacob, *Ind. Eng. Chem., Anal. Ed.*, 3, 371 (1931).

however, and the acidity of the total distillate was equal to 0.2 ml. of 0.1 *N* alkali. Portions of the distillate gave a slight turbidity with barium chloride.

Most investigators agree that distillation temperatures of 135°–140° C. with distillate volumes of 150–200 ml. should be sufficient to recover all the fluorine.

## METHODS

*A. Thorium Nitrate Titration.*—The “back-titration” procedure, applied without neutralization and concentration of distillates by Dable, Wichmann, and Bonnar and discussed last year, *This Journal*, 21, 468 (1938); 23, 303 (1940), has been used as a standard method by the Associate Referee. The only modification introduced has been the above-mentioned mixing in of 1 ml. of 0.1 per cent hydroxylamine hydrochloride solution to the distillate aliquot, before the dye is added, to eliminate possible traces of chlorine, and the use of a like quantity in the blank tube. As large an aliquot as practicable should be taken for the titration in order to avoid unnecessary multiplication of any titration error. To this end, 100 ml. long-form Nessler tubes are used wherever possible, with distillate aliquots up to 90 ml. However, there is an upper limit to the quantity of fluorine that can be titrated in a single tube, which is roughly about 50 micrograms for a 50 ml. Nessler tube and about 80 micrograms for the 100 ml. size. This is because introduction of excessive amounts of  $\text{Na}^+$  ion as standard sodium fluoride solution in the back titration develops a “salt effect” with attendant off shades and sluggish end points. The Associate Referee has noted an unexplained tendency to undertitrate slightly. The precision to be expected with the method can be illustrated by the series of results, shown in Table 2, where the varying quan-

TABLE 2.—Results showing precision of Method A

NO.	$\text{Th}(\text{NO}_3)_4$	RECOVERED	ADDED	ERROR
	ml.	micrograms	micrograms	micrograms
1	0.75	0.5	0.5	—
2	0.75	1.6	1.5	+0.1
3	1.25	6.5	6.4	+0.1
4	2.00	12.6	12.5	+0.1
5	1.75	14.0	15.0	−1.0
6	3.00	30.2	31.5	−1.3
7	4.50	49.1	50.5	−1.4
8	5.75	60.6	62.7	−2.1
9	5.50	68.5	68.8	−0.3
10	6.50	74.1	75.0	−0.9

tities of fluorine were added as unknowns directly to 100 ml. Nessler tubes.

John F. Weeks, Jr.\* submitted a similar series of results, in most of which the error was very small.

**B. Aluminum-Aluminon Method.**—The thorium nitrate procedure has come to be standard for the micro determination of fluorine. However, all analysts will agree that as a check upon results by this method an independent procedure of about equal scope and accuracy is desirable. This would likely be the only reason for developing a new method, since, according to Fahey,<sup>7</sup> nearly 20 have appeared.

The principle of the procedure was outlined by the Associate Referee last year, *This Journal*, 23, 303 (1941). As with all colorimetric fluorine methods the quantity of fluorine is derived from its bleaching effect upon a colored complex. This year effort was directed to the securing of reproducible and reasonably stable colors and to adapting these to photometric measurement in order to eliminate the personal error attendant upon visual judging of end points. Interferences were also investigated. In its present form, the method has been adapted with success only to the distillates from completely ashed samples, and in this respect it resembles the thorium nitrate procedure. As outlined here, the procedure has been adapted to the neutral wedge photometer<sup>8</sup> with a filter centered at 524 m $\mu$ , and a 100 mm. cell.

#### METHOD

##### REAGENTS

(1) *Distilled water.*—Redistil from alkaline permanganate, using all Pyrex apparatus.

(2) *Standard fluoride solution.*—Prepare a stock solution from pure NaF containing 1.0 gram of F per liter and preserve in a paraffined bottle. Prepare dilutions as needed, such as 1 ml. = 10 micrograms of F. Dilutions will hold several weeks in ordinary volumetric ware.

(3) *Aluminum-acetic acid mixture.*—Dissolve exactly 1 gram of purest  $\text{Al}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$  (99.5% assay, or better, A.C.S. or U.S.P. test) in water, transfer to a 1 liter volumetric flask, add 1–2 drops of  $\text{H}_2\text{SO}_4$ , and make to volume. Store the solution in Pyrex. (This is the stock Al solution.) *Mixture a*: 20 ml. of this stock Al solution + 25 ml. of glacial acetic acid made to 100 ml. *Mixture b*: An exact 1 + 1 dilution of Mixture a.

(4) *Gelatin solution.*—2%. Transfer to a beaker 2.00 grams of purest obtainable edible gelatin, add 30–40 ml. of water, allow to swell for a few minutes, place on the steam bath, and heat until dissolved. Flush into a 100 ml. volumetric flask with water, cool, and make to mark, using a drop or two of alcohol, if necessary, to dispel foam. The solution should be almost entirely clear. If it tends to gel upon standing, warm slightly on the steam bath. *Prepare fresh daily.*

(5) *Aluminon (aurintricarboxylic acid).*—35 mg. of the pure dye plus 200 ml. of saturated sodium acetate solution plus 50 ml. of glacial acetic acid per liter. Transfer the weighed dye to a beaker, dissolve in some of the sodium acetate solution plus a little water, rinse into a 1 liter volumetric flask, and add the remainder of the acetate solution and the glacial acid. Mix, and make to volume. (Commercial

\* Food and Drug Administration, New Orleans. Private communication.

<sup>7</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 362 (1939).

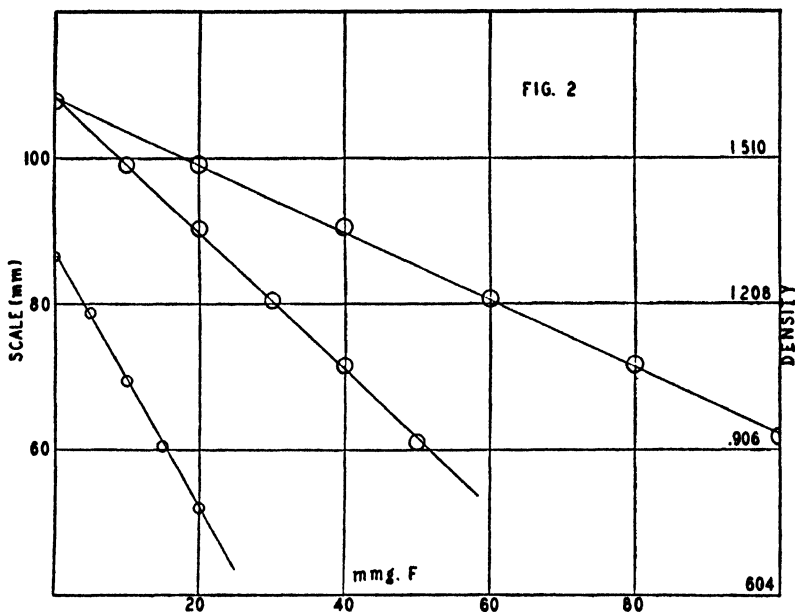
<sup>8</sup> Clifford and Brice, *Ibid.*, 12, 218 (1940); American Instrument Co., Silver Spring, Md., Bull. 2080.



samples of the dye vary widely in purity, and in working with an impure dye it will probably be necessary to increase the quantity used until the lake density for zero F is approximately 1.5 for the 0-100 microgram range. Holaday and Scherrer and Smith<sup>9</sup> have given procedures for the preparation of the pure product. Little increase in purity has been obtained by dissolving the impure dye in dilute alkali and reprecipitating with acid, although this procedure is of value in freeing the product from nitrites, which may remain in the dye as a contaminating by-product and cause bleaching of the standard dye solution. This solution is quite stable but a slight bleaching has been noted in about 6 weeks' time. However, if the bulk of it is stored in the cold, and only enough for a few days' work withdrawn at one time, it will keep almost indefinitely.

#### PREPARATION OF STANDARD CURVES

Three standard curves, covering three F ranges, have been used, respectively, 0-100, 0-50, and 0-20 micrograms. Directions for the preparation of one curve only are given as manipulative details are identical in the three cases. All measurements are exact. For the 0-100 range proceed as follows: Into six 100 ml. glass-stoppered,



graduated, Pyrex cylinders introduce, respectively, 0, 20, 40, 60, 80, and 100 micrograms of F. Make to exactly 75 ml., then add 5 ml. reagent 3a and 10 ml. reagent 4. Mix gently by inverting several times, and add 10 ml. reagent 5. Stopper *tightly*, mix by inverting gently 4-5 times, and immerse to the neck in a water bath at 80° C. for 30 minutes. Remove, cool under the tap to room temperature, and allow to stand for 15 minutes after removal from the bath. Do not over-cool below 20° C. Fill into a 4" cell and read in the photometer within 20 minutes, using filter No. 52. Plot scale readings against quantity of F to obtain the standard curve or reduce the data by the method of least squares to obtain a factor giving micrograms of F in

<sup>9</sup> Holaday, *J. Am. Chem. Soc.*, 62, 989 (1940); Scherrer and Smith, *J. Research, NBS*, 21, 113 (1938) R.P. 1118.

terms of scale reading. Proceed similarly for the other ranges, using intervals of 10 micrograms for the 0-50, and of 5 micrograms for the 0-20 range. Typical curves are given in Figure 2. Volumes of reagents, etc., for the three ranges are summarized in Table 3.

TABLE 3.—*Volumes of reagents for preparation of standard curves*

RANGE	SIZE OF CYLINDER	INITIAL VOLUME	REAGENT 3	REAGENT 4	REAGENT 5
	ml.	ml.	ml.	ml.	ml.
0-100	100	75	5 3a	10	10
0- 50	50	35	5 3b	5	5
0- 20	25	20	1 3a	2	2

#### DETERMINATION

Proceed as directed for preparation of standards with aliquots of the distillate, or if aliquots larger than those listed under "Initial Volume" are desired, neutralize to phenolphthalein or preferably *p*-nitrophenol (0.5% alcoholic solution), evaporate down in platinum, and transfer to the proper sized cylinder. The total distillate can be used in this manner.

The lake colors are more intense when hot, and a certain time is required for the intensities to adjust to room temperature. After this cooling period they fade slowly, but this fading is hardly significant within a period of 30 minutes. Turbidities will cause high photometric readings and low results, but if the alkali used for neutralization is preserved silicate free and evaporations are made in platinum, these are not excessive. The Associate Referee has used the same 0.05 *N* sodium hydroxide (preserved in a paraffined bottle) for neutralization as that used in the titration of distillates for the back-titration procedure (Method A). Formerly a turbidity correction was made by bleaching the lake with a few ml. of nitric acid and subtracting any positive difference between this reading and the average "turbidity" readings of the standards, so treated, from the reading of the lake color. This procedure has been found to be unnecessary in ordinary work. The buffer mixture adjusts to a pH of 4.2 and will protect against any reasonable amount of distillate acid or salts and excess alkali resulting from its neutralization. Cylinders should be of Pyrex to minimize breakage and must be carefully checked for volume. At least they must be of the same type of glass, as certain types contribute more silica and aluminum to the color mixture by leaching. The proportion of aluminum to dye has been so adjusted that the photometric density ( $-\log T$ ) of the lake colors are proportional to the quantity of fluorine over, and slightly beyond, the given range. If too little aluminum is used the curve will be "concave" and if there is scarcity of dye the curvature will be reversed. Interferences in the aluminon method are phosphates, nitrates, nitrites, and other reducing substances such as sulfur dioxide. Sulfates do not interfere. Free chlorine interferes by bleaching

the lake color and possible traces of chlorine are discharged as in the titration procedure by adding 1 ml. of 0.1 per cent hydroxylamine hydrochloride to the aliquot before neutralization and evaporation, or directly to the cylinders if the distillate is not concentrated. The various metallic interferences would not be expected in a fluorine distillate.

Accuracy of the method with the varying ranges is illustrated in Table 4, where the fluorine was added as unknowns directly to the cylinders.

TABLE 4.—Accuracy (in micrograms) of method at varying ranges

RANGE	ADDED	FOUND	ERROR	ADDED	FOUND	ERROR
0- 20	2.2	2.1	+0.1	13.7	13.7	—
	2.5	2.0	-0.5	16.3	16.8	+0.5
	5.2	5.0	-0.2	20.0	19.3	-0.7
0- 50	12.5	12.1	-0.4	29.5	29.1	-0.4
	20.5	20.4	-0.1	30.3	30.8	+0.5
	22.5	21.0	-1.5	49.5	49.5	—
0-100	10.5	8.1	-2.4	82.5	82.6	+0.1
	47.5	48.3	+0.8	89.5	89.6	+0.1
	52.5	53.5	+1.0	99.5	98.2	-1.3

#### GENERAL

The subject of sample preparation needs further investigation. Both methods require the nearly complete destruction of organic matter, Dahle, *This Journal*, 22, 338 (1939), although the presence of a certain amount of carbon in the distilling flask does no harm. An ashing procedure for foods postulates the use of fixatives, and several have been suggested. Dahle, *This Journal*, 18, 194 (1935); 19, 228 (1936), investigated the use of lime and aluminum, but he favors lime because aluminum retards the distillation of fluorine. Winter, *Ibid.*, 19, 359 (1936), used magnesium acetate in the ashing of plant materials, and MacIntire and Hammond, *Ibid.*, 22, 231 (1939) used magnesium peroxide with success in the case of organics. Rempel<sup>10</sup> reports calcium oxide to be much more effective than magnesium oxide and finds that sodium carbonate alone will not retain fluorine above 450° C. The Associate Referee has found that losses can be detected even at 400° C. in the case of sodium carbonate. It thus appears that the alkali earths, especially calcium, show most promise as fluorine fixatives. J. F. Weeks, Jr. (private communication) has had success with a fixative suspension prepared by slaking 25 grams of low fluorine lime\* (<2 p.p.m.) with 1 liter of water, and using up to 25 ml. for ashings, wherein temperatures as high as 750° C. were used. The Associate Referee has prepared a fluorine-free lime suspension as follows: 56 grams (1 mol)

<sup>10</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 378 (1939).

\* Manufactured by Victor Chemical Co., Chicago, Ill.

of low-fluorine lime was carefully slaked and 250 ml. of 60 per cent perchloric acid was added slowly and with stirring. The solution was boiled down over a free flame to copious fumes of acid, then 300 ml. of water was added and the solution was boiled down again. The dilution and boiling down were repeated twice more, and the solution was diluted somewhat and filtered through a large fritted glass filter to remove a precipitate of gelatinous silica. The filtered solution was then poured slowly into 1 liter of 10 per cent sodium hydroxide, and the precipitate was allowed to settle. The supernatant liquid was siphoned off, and the precipitate was transferred to large (500 ml.) centrifuge bottles and washed 5 times with water by means of the centrifuge, the substrate being shaken up thoroughly each time. The precipitates were then shaken into suspensions, combined, made to 2 liters, and preserved in paraffined bottles; 100 ml. of this suspension gave no perceptible blank.

Small quantities of this suspension (2-5 ml.) will fix 250 micrograms of fluorine at 600° C. when no organic matter is present. However, when 5 ml. of the suspension was added to a solution of sucrose sirup containing 5 grams of sugar and an added 250 micrograms of fluorine, and the mixture was evaporated and charred over a low flame before ashing, only 17 per cent was recovered. Under the same conditions 20 ml. of the lime suspension returned 99 per cent. When ashing was at 650°, 20 ml. returned 90 per cent.

With the smaller quantity of fixative a piece of moist blue litmus held in the fumes during the charring process proved these vapors to be quite acid. They were much less so with larger quantities of fixative solution. This demonstrates the necessity of enough fixative to maintain an organic sample in an alkaline condition at all times during the drying and preliminary oxidation.

The danger of loss of fluorine with certain fatty and proteinaceous materials through improper "wetting" by a fixative solution has been pointed out by Dahle, *This Journal*, 19, 228 (1936). Cox et al.<sup>11</sup> point out the questionable value of fluorine analyses of products that must be ashed. The Associate Referee believes that in dealing with organic materials much of this danger may be eliminated by a double distillation, Wichmann and Dahle, *This Journal*, 16, 620 (1933); Eberz et al., *loc. cit.*, first from sulfuric, then perchloric acid. The first distillate is treated with an excess of the lime-water fixative, evaporated to *dryness*, ashed for 1-2 hours at 550-600° C., taken up, and redistilled. The size of the sample is limited by the amount that will not foam over or char in the distilling flask during the distillation with sulfuric acid, and here larger flasks and more distilling acid may sometimes be indicated. Adequate amounts of silver sulfate or perchlorate should be used to prevent the evolution of hydrochloric acid. The double distillation procedure is especially indicated with materials

<sup>11</sup> *J. Dental Res.*, 18, 486 (1939).

of high phosphate content in order to minimize distillation of phosphoric acid, which interferes in the determination, Reynolds, *loc cit.*, and Churchill, Bridges, and Rowley.<sup>12</sup>

Sulfuric acid cannot be used in the first distillation with products such as bone or food phosphates, containing a large proportion of calcium, because of the precipitation of calcium sulfate in the distilling flask. Here perchloric acid may often be used, and with 20 ml. the Associate Referee has handled 5 gram samples of raw bone. If temperatures are kept below 150° C. danger of explosion in the distillation is minimized.

Although losses of the order of 3–6 per cent may attend the double distillation procedure because of incomplete evolution of fluorine, in many cases it may still be the safest procedure to apply.

Methods A and B were used jointly on a number of samples of foods and biological material with fairly satisfactory agreement. The procedure usually is as follows: A 50 ml. aliquot of the final distillate is titrated with 0.05 *N* sodium hydroxide and reserved. The back-titration procedure is then applied to another aliquot, the size depending upon the nature of the material being analyzed and its expected fluorine content. A satisfactory figure for the titration method determines the range and aliquot size to be used in Method B, and it is best to choose these so that the reading will fall in the higher portion of the chosen range. For low quantities of fluorine the 50 ml. of distillate used for the acidity determination is joined to any remaining distillate and evaporated down in platinum for transfer to the proper sized cylinder. The indicator used in the titration must be colorless at the pH (4.2) of the colorimetric method and hence the specified alizarin is unsuitable. The Associate Referee finds 1–2 drops of a 0.5 per cent alcoholic solution of *p*-nitrophenol very satisfactory and it yields a good end point and no turbidity. Phenolphthalein is next choice. The use of a little hydroxylamine hydrochloride to discharge traces of chlorine when perchloric acid has been used has already been mentioned. Method B can be used with sulfuric acid as the distilling medium, where its use is contraindicated in Method A.

Certain incidental precautions may be mentioned. It is best to use platinum for all evaporations and ashings in the micro procedure as porcelain has been found to contribute small quantities of fluorine. McClure<sup>13</sup> notes further that some material, presumably dissolved aluminum silicate, causes low results in the titration when alkaline solutions are evaporated in porcelain. To avoid contamination in the muffle dishes should be covered, and Pyrex petri dishes are suitable for this purpose. Filtrations should be made through carefully cleaned fritted-glass filters to avoid contamination with hydrofluoric acid-treated filter papers; 150 ml. of a solution containing 0.133 microgram of fluorine per ml. titrated

<sup>12</sup> *Ind. Eng. Chem., Anal. Ed.*, 9, 222 (1937).

<sup>13</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 171 (1939); *Nat. Inst. Health Bull.* 172 (1939).

0.183 microgram per ml. after filtering once through a C.S.&S. 12  $\frac{1}{2}$  cm. folded filter, taking up in all, 7.4 micrograms.

Additional work should be directed towards the working out of proper methods of sample preparation for various type products, and the existing methods should be given further collaborative study. Spray residue procedures have not been mentioned, but here again further work is indicated.

It is recommended that work on fluorine be continued.

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### REPORT ON LEAD

By P. A. CLIFFORD (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Work was confined to an adaptation of the rapid dithizone method restricted to apples and pears, to conditions covering a higher range, *Methods of Analysis, A.O.A.C.*, 1940, 407, 30-32. The changes involved were adopted and published in *This Journal*, 24, 80 (1941).

Attention is directed to an error in item No. 7, page 81, of the above reference. The expression "20 ml." should read "30 ml."

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No report on mercury was given by the associate referee.

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### REPORT ON SELENIUM

By A. K. KLEIN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

While the tentative method for the determination of selenium in foods developed by the previous associate referee and coworkers, *Methods of Analysis, A.O.A.C.*, 1940, 417, yields uniformly reliable results, the present Associate Referee undertook further studies with a view to its possible improvement, especially for the determination of extremely small quantities. The essential chemical principles were not altered. Certain refinements in apparatus and manipulation are presented because they seem to contribute increased accuracy in estimating this element in quantities from 1 to 10 micrograms.

Other studies were also undertaken in accordance with the suggestions outlined by the Referee on Metals in Foods in his report for 1939, *This Journal*, 22, 316 (1939). These suggestions include possible refinement of the proposed tentative method and certain colorimetric procedures.

## EXPERIMENTAL

### *The Tentative Method*

(1) *Sample Preparation.*—Since it had been established by previous workers that all steps in the procedure except possibly the digestion of the sample are quantitative, a rather exhaustive study of this part of the method was made.

Selenium as potassium selenite, in the following proportions, was digested with nitric-sulfuric acid and mercuric oxide until sulfur trioxide had been evolved for 10 minutes, a treatment more severe than that used in the tentative method or recommended by the Associate Referee.

<i>Se added</i> <i>micrograms</i>	<i>Se found</i> <i>micrograms</i>	<i>Recovery</i> <i>per cent</i>
50.6	50.7	100.2
460.2	455.7	99.0
920.4*	907.4	98.6
1840.8†	1823.0	99.0

\* Half aliquot of distillate used.

† One-quarter aliquot of distillate used.

All the selenium recoveries discussed under "The Tentative Method" were obtained by the refinement of the present tentative method, to be described later. These recoveries indicate that selenium, in the oxidized form with mercury as fixative, is relatively stable. However, since slight losses seem to occur with excessive heating, prolonged digestion is to be avoided.

Following this experiment, varying quantities of the element as the selenite were added to 10 grams of oven-dried and well ground orange peel, tested and found to be free of selenium. The digestion was conducted in an all-glass apparatus, consisting of a round-bottomed flask and a modified Soxhlet with no overflow. After the addition of the acids and mercury, the flask was placed on an active steam bath for 30 minutes. The copiously evolved oxides of nitrogen, usually permitted to escape, were collected in an excess of a cold solution of sodium hydroxide (1 + 2). In no instance was selenium detected in the alkali even when more than 1,000 micrograms was originally present in the sample. It may be assumed, therefore, that selenium is not volatilized during the heating on the steam bath, a preliminary treatment recommended by the Associate Referee for digesting samples.

A condenser was then affixed to the Soxhlet, and digestion was continued, with heat from a burner, to incipient fumes of sulfur trioxide. The nitric acid, which usually boils away, collected in the Soxhlet and was returned to the digest. Heating to incipient fumes was repeated. The color of the digest was then of a light straw yellow, indicating complete or almost complete digestion.

The acid in the Soxhlet contained no selenium when no more than 1,000

micrograms was present in the digest. However, when portions of 10 grams of *Astragalus Pectinatus* containing 1,900 p.p.m. of the element or 19,000 micrograms was similarly treated, 8.4 micrograms in one case and 3.4 in another were found in the nitric acid fraction. In proportion to the rather large quantities of selenium originally present, the amounts that would have been lost had open beakers been used are almost negligible (at most 0.04 per cent of the total). When a mixture of 1 gram of the vetch and 9 grams of the fruit rind was similarly treated, only 1.2 micrograms of selenium was found in the acid fraction. In the interest of precision, therefore, it may be well to use smaller samples in analyzing material comparatively rich in the element.

The following data were obtained from digests treated as just described:

	<i>Se added</i> micrograms	<i>Se found</i> micrograms	<i>Recovery</i> per cent
1.	10.0	10.0	100.0
2.	100.0	99.3	99.3
3.	1000.0*	990.0	99.0

\* Half distillate for final Se estimation.

Recovery No. 2 is within the titration error, but the 1 per cent variation in No. 3 is slightly greater than the final error of estimation. Since no selenium was found in either the nitric acid fraction or the alkali reservoir, the slight loss may be attributed to subsequent transfer of material.

The digestion experiments are described in detail because some chemists question the ability of mercury to fix selenium during a hot acid digestion of organic matter. For most products the usual digestion is effective in decomposing organic selenium into inorganic acids capable, subsequently, of being separated by distillation as the volatile bromide. However, L. Greathouse (private communication) observed that certain vetches and seedlings comparatively rich in selenium are particularly refractory and require more than the usual nitric-sulfuric acid treatment. Accordingly, following the usual digestion and two distillations, the residues from 10 grams of the *Astragalus Pectinatus*, previously referred to, were subjected to a vigorous perchloric acid treatment. One residue did yield an additional 23 micrograms of selenium, another 28. Again, if the 1,900 p.p.m. originally present are considered the residual selenium, averaging 2.5 p.p.m., would seem insignificant. From the residue of 1 gram of vetch mixed with 9 grams of dried fruit rind only 0.6 microgram of selenium was recovered. Therefore, by restricting the size of sample even refractory substances rich in selenium are amenable to the usual digestion treatment. When 10 grams of sorgho seed containing 45 p.p.m. of the element was digested simply with nitric acid, no selenium was found in either the collected acid fraction or the residue after distillation. Therefore it is evident that the closed system used for the previous studies is not re-



quired in actual practice. Open beakers may be used with impunity provided the sample does not contain more than 1,000 micrograms of selenium.

A variety of products was analyzed in open beakers with the results shown in Table 1.

TABLE 1.—*Results obtained by use of open beakers*

PRODUCT	WEIGHT	Se ADDED	Se FOUND	RECOVERY	
	grams	micrograms		micrograms	per cent
Eggs	43		43.9		
		43	85.8	41.9	97.4
		86	126.0	82.1	95.5
		129	168.0	124.1	96.2
Beef	50		6.4	Average 6.7	
			7.0		
			6.6		
		10	16.9	10.2*	102
		15	22.0	15.3*	102
		20	26.9	20.2*	101
Grape Fruit Rind	15	25	24.2	24.2	96.8
		50	50.8	50.8	101.6
		100	97.6	97.6	97.6
Sucrose	15	12.50	12.7	12.7	101.6
		5.75	5.6	5.6	97.4
		1.50	1.5	1.5	100.0
		4.75	4.7	4.70	99.0
		5.75	5.90	5.90	102.6

\* The average figure 6.7 was used in evaluating these results. The eggs used as base were taken from a well mixed batch of a dozen freshly shelled eggs. Similarly, the 50 grams of beef came from 2 pounds of freshly ground and well mixed meat.

These recoveries (Table 1) indicate that the procedure for sample preparation developed by the previous associate referee is in the main satisfactory. Products like eggs and meat are only partially digested by the suggested treatment, since fats are oxidized with extreme difficulty by nitric acid. The work of Gortner,<sup>1</sup> among others, indicates that selenium is contained in the protein and not the fat fraction, but in the present studies no attempt was made to separate the fat from the bulk of the acid digest. Some bumping was encountered during the subsequent distillation, but the operation was possible, although the recoveries were a bit low in these instances. Therefore, if desired, fats may be removed in part by chilling the digest and filtering on a large sintered-glass filter.

(2) *Distillation and Reduction*.—All investigators are agreed that selenium can be isolated most effectively from interfering material by dis-

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 198 (1939).

tillation as the volatile bromide. Under the imposed conditions only arsenic, antimony, tin, germanium, and selenium distil, and of these elements selenium is the only one that is reduced to the insoluble element by the addition of sulfur dioxide.

Accordingly, except for reducing the quantity of hydrobromic acid-bromine needed for the distillation, this part of the method was not altered. Some attention should be given to the ratio of added hydrobromic acid to the water contained in the digest. If the acid is present in excess of 42 per cent by volume (composition of the constant boiling reagent) owing to dehydration by sulfuric acid, it will distil first as the gas. A workable rule is to add 25 ml. of water to the digest for every 25 ml. of bromine reagent, in addition to 25 ml. of water to effect the transfer.

The reduction of excess bromine in the distillate, together with the precipitation of selenium by sulfur dioxide, is quite regular and requires no comment.

(3) *Filtration and Titration.*—It is in these two steps of the tentative method that possible refinements or changes in apparatus and technic are presented, although even here the essential principles are not altered. In particular, instead of the use of a Gooch for collecting the precipitated selenium, the smaller sintered-glass filtration vessel illustrated in Figure 1 is suggested. With its use, five 1 ml. portions of water are sufficient to rinse the precipitation flask and filtration pad free of excess acid and reducing substances. And by employing a dilute solution of hydrobromic acid-bromine, needed for the conversion of selenium to selenious acid, the addition of dilute phenol alone is sufficient to neutralize the excess of bromine, although heating is required subsequently to complete the reaction. The selenious acid is collected directly in the titration tube. It is possible to make a quantitative transfer with only 5–6 ml. of liquid reagents.

That the use of small volumes of transfer liquid yields quantitative recoveries even for comparatively large quantities of the element is shown by the following experiment: Three 5 ml. portions of selenious acid, each equivalent to 460.2 micrograms of the element, were distilled, precipitated, washed, and titrated in the tube. The recoveries were 456.7, 458.7, and 460.7 micrograms, averaging 458.7 micrograms.

As in the tentative method, the Norris-Fay<sup>2</sup> thiosulfate-iodine titration was used for the final evaluation of the element. The following equation describes the essential reaction:



Standard thiosulfate is added in excess, and the titration is completed with standard iodine, starch indicator being used. A somewhat optimum pH must obtain for best results; too high a pH causes an incomplete re-

<sup>2</sup> *Am. Chem. J.*, 18, 705 (1896).

action between selenious acid and thiosulfate. When the acid containing the selenium was buffered to a pH of 4 by sodium acetate prior to the addition of thiosulfate, no reaction took place. Too much acid tends to effect a recurrent end point, due probably to the slow formation of iodine from the iodide present in the standard iodine reagent. The acidity contributed by the dilute hydrobromic acid-bromine reagent seems to provide a satisfactory medium.

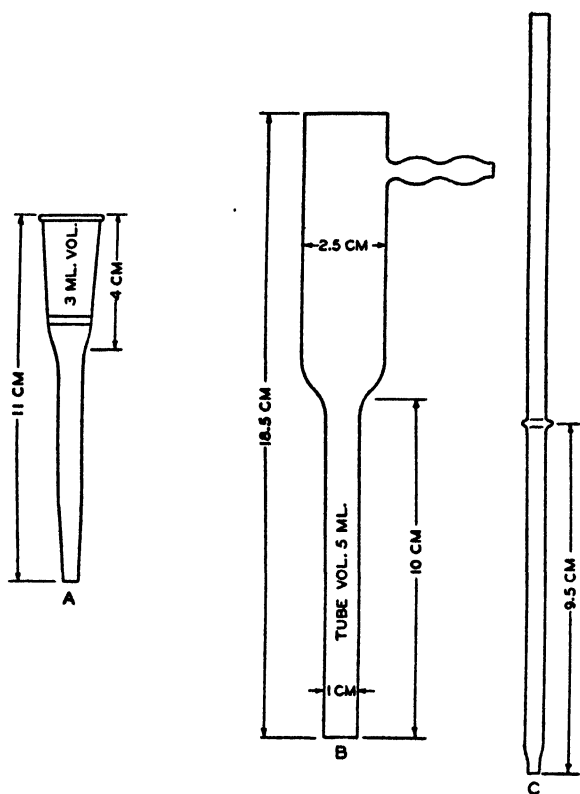


FIG. 1.—FILTRATION AND TITRATION TUBES AND PIPET.

The titration tube, by virtue of its Nessler-like construction, is especially helpful in estimating selenium in the ranges of 1–10 micrograms, *This Journal*, 22, 441 (1939). For these amounts 0.001 *N* standard solution should be used. By viewing the color of the solution down the length of the tube against the surface of unglazed white opal glass, differences in color produced by increments of 0.01 ml. of the dilute reagents can be discerned, especially if the volume is restricted to 7–8 ml. An excess of 0.05 ml. of 0.001 *N* iodine produces an unmistakable blue color. An additional aid in determining the end point is the use of titrating tubes with flat, fused, optical bases; painting the outside walls of the instrument black also helps.

The following experiment indicates that stoichiometric relations obtain for even 0.001 *N* solutions. A thiosulfate solution standardized against exactly 0.1 *N* potassium dichromate was found to be 0.1028 *N*. Ten ml. of the solution was diluted to a liter. The resulting thiosulfate should be 0.001 *N* with a conversion factor of 1.028. Likewise, an iodine solution standardized against the stronger thiosulfate was found to be 0.0985 *N*. Exactly 10.15 ml. of the reagent was diluted to a liter after the addition of potassium iodide as a stabilizer. This solution should be exactly 0.001 *N*. Cross titration of the dilute solutions yielded the following data:

Thiosulfate ml.	Iodine ml.	Iodine Thiosulfate
2.94	3.00	1.020
3.43	3.50	1.020

Thus, the observed conversion factor, 1.020, differs only by 0.008 from the calculated value of 1.028, or within 0.8 per cent.

(4) *Titration of Thiosulfate against Selenious Acid.*—

Se micrograms	.001 <i>N</i> Thio- sulfate ml.	Corrected ml. × 1.02	0.001 <i>N</i> I <sub>2</sub> ml.	Net Thio- sulfate ml.	Micrograms Se Net thiosulfate
50	4.45	4.54	2.00	2.54	19.7
60	5.10	5.20	2.17	3.03	19.8

The selenious acid was prepared by dissolving an accurately weighed amount of the element in hydrobromic acid and bromine, almost neutralizing the latter with sulfur dioxide, and completing the reduction with phenol.

The stoichiometric equivalent of 1 ml. of exactly 0.001 *N* thiosulfate is 19.8 micrograms of selenium, the value approached in the actual experiment.

The method to follow, applicable especially for selenium in the range 1–25 micrograms, is also submitted for the determination of the element in larger quantities.

# SELENIUM

## Modified Tentative Method

### 1

#### REAGENTS

(a) *Sulfuric-nitric acid.*—To 50 ml. of H<sub>2</sub>SO<sub>4</sub> add 100 ml. of HNO<sub>3</sub>. Cool the mixture before using.

(b) *Mercuric oxide fixative.*—Dissolve the oxide in HNO<sub>3</sub> in the proportion of 5 grams to 100 ml. of the acid.

(c) *Hydrobromic acid-bromine.*—Concentrated. Mix 10 ml. of Br with 990 ml. of constant boiling HBr.

(d) *Sulfur dioxide.*—The gas supplied in commercial cylinders is free of Se.

(e) *Hydroxylamine hydrochloride.*—10% W/V in water.

(f) *Hydrobromic acid-bromine*.—Dilute. To 5 ml. of HBr add 10 ml. of saturated Br water and dilute to 100 ml. with water.

(g) *Phenol*.—5% W/V in water.

(h) *Standard sodium thiosulfate*.—Prepare from accurately standardized 0.1 N reagent. Before adjusting to final volume add 5 ml. of amyl alcohol per liter and shake vigorously. 1 ml. of 0.001 N thiosulfate is equivalent to approximately 20 micrograms of Se. (For the estimation of Se in quantities greater than 50–75 micrograms, proportionately stronger concentrations of thiosulfate are required.)

(i) *Standard iodine*.—Prepare from 0.1 N reagent. Before final dilution add KI in the proportion of 20 grams per liter. Dilute to like normality of thiosulfate.

(j) *Soluble starch indicator*.—0.5 W/V. (Merck's reagent has been used with good results.)

## 2

## APPARATUS

An all-glass distillation outfit consisting of 250 ml. round-bottomed flask, still head, thermometer, and condenser with dipping end.

## 3

## DETERMINATION

Place 5–10 grams (dry weight) of the sample in a 600 ml. Pyrex beaker and add 150 ml. of the  $\text{H}_2\text{SO}_4$ - $\text{HNO}_3$  followed by 10 ml. of the mercuric oxide fixative. If the product is rich in Se, use 1 gram of representative material. Immediately mix thoroughly and place on the steam bath for 30 minutes, stirring intermittently. Then heat with the burner (not full flame) until the product lightens and then turns brown. Remove the flame, cool, and after adding 10 ml. of the concentrated  $\text{HNO}_3$ , again heat until the first brown appears. Repeat this operation at least twice and then heat until the solution turns a distinct brown (not black) or until  $\text{SO}_2$  fumes appear. (It is imperative that the excess  $\text{HNO}_3$  be expelled and that the organic matter be sufficiently oxidized so that the bromine reagent subsequently added is not reduced, but prolonged fuming to  $\text{SO}_2$  is to be avoided.) As such products as molasses and honey, principally sugars, react vigorously with  $\text{HNO}_3$ , remove such samples from the steam bath until the first reaction subsides and then proceed in the usual manner.

Cool the digest and transfer with two 25 ml. portions of water to the distilling flask. Rinse the beaker carefully with 25 ml. of the HBr-Br (50 ml. for products of high Se content), and add to the cooled digest and washings. After swirling the flask, distil until the temperature of distillation reaches  $130^\circ\text{C}$ . into a 125 ml. Erlenmeyer flask containing 5 ml. of HBr and surrounded by cold water. (The volume of distillate is about 75 ml. when 25 ml. of the HBr has been added. Free bromine should distil at the beginning, indicating an excess of the reagent. If this is not the case, stop the distillation, cool, and add an additional 10 ml. of the reagent. This contingency arises only with insufficient digestion of sample.) If the sample is a vetch or seedling rich in Se, heat the residue to the first fumes of  $\text{SO}_2$ , cool, add 5 ml. portions of  $\text{HClO}_4$ , and heat to fuming. Then cool, transfer to the distillation flask with two 25 ml. portions of water and finally with 25 ml. of the HBr-Br and distil. Add the distillate to the original distillate. (The combined distillate may be diluted with water to suitable volume and appropriate aliquots drawn for subsequent analyses.)

Filter off with suction waxes and other insoluble material in the distillate on asbestos and rinse flask and pad with 2–5 ml. portions of water. Saturate the filtrate with  $\text{SO}_2$  and after adding 1 ml. of the  $\text{NH}_4\text{OH}\cdot\text{HCl}$ , cap the flasks with watch-glasses and set on steam bath for 30 minutes. Place in cold water for a like period and then with suction collect the Se on an asbestos pad contained in the filtration vessel, Figure 1. Rinse the precipitation flask and pad with 5 successive 1 ml. por-

tions of water from a pipet, and then hold the mouth of the flask before an air vent to remove the last traces of  $\text{SO}_2$ .

Insert filtration vessel into titrating tube and dissolve the Se with 1 ml. of the dilute  $\text{HBr-Br}$ , first adding the reagent from a pipet to the flask and then transferring carefully to the pad. When the Se has dissolved, apply gentle suction and repeat the operation with an additional 1 ml. of the dilute  $\text{HBr-Br}$ . Finally rinse flask and pad with 3 successive 1 ml. portions of water, collecting the filtrate before each addition. 2 ml. of the dilute  $\text{HBr-Br}$  reagent is sufficient for Se up to 500 micrograms. When more is present, use proportionately more reagent and rinse water.

Agitate the filtrate with pipet stirrer and dispel excess Br with 3 drops of the phenol. Using the stirrer as a pipet, rinse the walls of the vessel several times with the solution to neutralize every trace of Br. Immerse the titrating tube up to two-thirds of its length in hot water for 5 minutes, stirring intermittently. (Heating is required to complete the reaction between the Br and phenol.) Then place the vessel in cold water for at least 5 minutes. (The Norris-Fay titration works best when the solution is below  $25^\circ \text{C}$ .)

Using the original precipitate of Se as a guide, add at least a 50% excess of the appropriate strength of the standard thiosulfate reagent and 3 drops of the starch indicator. After stirring, add the standard I until a permanent blue color appears. If less than 1 ml. of I is required, add sufficient thiosulfate so that at least 1 ml. of I is required. Then titrate to a colorless endpoint with thiosulfate, adding the reagent in increments of 0.01 ml. as the endpoint is approached.

## 4

## CALCULATIONS AND STANDARDIZATION

## (Cross Titration)

To 2 ml. of  $\text{HBr}$  (5+95) add 3 ml. of water and 3 ml. of standard I. Titrate with the standard thiosulfate and toward the end add 3 drops of the starch indicator. Complete the titration as directed in the sample determination and obtain the thiosulfate equivalent of the iodine.

## 5

## STANDARDIZATION OF THIOSULFATE

Add 2 ml. of the dilute  $\text{HBr}$  to an appropriate volume of standard Se solution and after the addition of 50% excess thiosulfate continue the titration in the usual manner. Obtain the Se equivalent of the thiosulfate.

## 6

## SAMPLE CALCULATION

Multiply the net thiosulfate sample titer in ml. by the Se equivalent to obtain the amount of Se in the sample.

The data in Table 2 indicate the recoveries that may be obtained if the suggested modifications of the tentative method are followed. The recovery experiments begin with the distillation of the element, added as selenious acid.

*Electrometric Titration*

J. A. Matthews, *This Journal*, 20, 197 (1937), using an electrometric titration of selenite solutions, reported results with a standard deviation of  $\pm 0.2$  microgram of selenium within the range of 0-10 micrograms. Standard thiosulfate is added in excess and the titration is completed with standard iodine until a standard galvanometer deflection is obtained.

In Matthews' work the volume finally titrated approximated 25 ml. Anticipating more accurate recoveries with the diminution in volume, the Associate Referee and Matthews redesigned the cell and electrodes so that the titration could be completed in 5 ml. volumes. The anticipated recoveries were realized. Selenium in the range of 1-10 micrograms was distilled, isolated, and titrated electrometrically with an average deviation of  $\pm 0.04$  microgram.

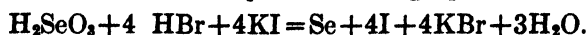
TABLE 2.—*Recoveries of Se by modified method*

Se ADDED	Se FOUND	DEVIATION
micrograms	micrograms	
None	0.10	+0.10
None	0.10	+0.10
1	0.9	-0.10
1	1.1	+0.10
2	2.1	+0.10
2	1.9	-0.10
3	3.1	+0.10
3	3.0	0.00
4	4.1	+0.10
4	4.1	+0.10
5	5.1	+0.10
5	5.1	+0.10
6	5.8	-0.20
6	5.9	-0.10
7	7.2	+0.20
7	7.0	0.00
8	8.0	0.00
8	8.0	0.00
9	9.0	0.00
9	8.9	-0.10
10	10.0	0.00
10	10.2	+0.20
Av. deviation = 0.09 microgram		

While this procedure is capable of giving very satisfactory results, it is somewhat tedious and painstaking, and the analyst has only the final deflection of the galvanometer as a guide. The caution demanded makes for slowness; in addition, the apparatus requires frequent adjustment and standardization. Therefore for ordinary work, which demands only moderate accuracy, it would seem that the starch iodometric procedure would be preferred. The data presented in Tables 3 and 4 indicate that this method can give very satisfactory results.

#### *Liberated Iodine Titration*

A short study was made of a micro volumetric method for the determination of selenium illustrated by the following equation:



This reaction has long been used as a gravimetric method for macro quantities of the element. Many attempts have been made to use it as a volumetric procedure, but no variation has met with general approval. The precipitated selenium occludes particles of iodine, preventing a correct titration with thiosulfate.

TABLE 3.—*Titration of selenite as "unknowns"*

Se ADDED	Se FOUND	DEVIATION
<i>micrograms</i>	<i>micrograms</i>	
2.10	2.11	+0.01
0.72	0.75	+0.03
0.44	0.47	+0.03
2.50	2.49	-0.01
3.16	3.14	-0.02
Av. deviation = 0.02 microgram		

For micro quantities, however, no occlusion was noted, but the yellow color contributed by the selenium persistently hindered visual inspection of the blue starch-iodine end point. Subsequent titration with thiosulfate did not change the blue to a colorless end point, but rather from a cloudy blue to varying shades of yellow, depending upon the amount of selenium present.

TABLE 4.—*Recoveries involving distillation, precipitation, etc.*

Se ADDED	Se FOUND	DEVIATION
<i>micrograms</i>	<i>micrograms</i>	
1	0.95	-0.05
2	2.02	+0.02
3	3.00	0.00
4	4.07	+0.07
5	4.95	-0.05
6	5.99	-0.01
7	7.04	+0.04
8	8.04	+0.04
9	9.08	+0.08
10	9.99	-0.01
Av. deviation = $\pm 0.04$ microgram		

Fifteen titrations of selenite solutions are presented in Table 5. The average deviation is  $\pm 0.3$  microgram. While the results are not so nearly accurate as those obtained by the Norris-Fay titration, they are, nevertheless, not seriously in error, and since the procedure requires only one standard solution, namely, thiosulfate, some analysts may consider it satisfactory.



TABLE 5.—*Titrations of selenite solutions*

Se ADDED	Se FOUND	DEVIATION
micrograms	micrograms	
4.10	4.25	+0.15
2.00	1.54	+0.46
2.40	1.93	-0.47
6.50	5.98	-0.52
5.00	5.40	+0.40
5.00	4.80	-0.20
8.00	8.10	+0.10
22.50	22.60	+0.10
12.00	12.60	+0.60
17.5	17.80	+0.30

*Colorimetric Methods*

(1) *Extraction of Selenium with Carbon Disulfide and Colorimetric Evaluation of Extract.*—Amorphous selenium, the red variety ordinarily obtained by reducing selenites with sulfur dioxide, is soluble in carbon disulfide. When the element exists as a suspension, however, a wetting agent, like ether, must also be added to effect complete solution.

Accordingly, quantities of selenium from 0 to 300 micrograms were precipitated in Pyrex extraction funnels from hydrobromic acid-bromine solution in the usual manner. Water was next added until a specific gravity of 1.10 was obtained. The suspension was then shaken with 10 ml. of carbon disulfide-ether mixture (4 parts carbon disulfide to 1 ethyl ether by volume). The extract was drained through a pledget of cotton into a 4 inch glass absorption cell and the resultant color was evaluated in the neutral wedge photometer,<sup>3</sup> violet filter No. 42 being used. (The spectrophotometric transmission curve of selenium in the carbon disulfide-ether solution demonstrated the need for this filter.)

Chart 1 illustrates the results. A fairly straight line function obtains when mm. wedge readings are plotted against micrograms of selenium. However, 300 micrograms of the element contribute a density corresponding to only 58.5 mm. net scale divisions, or only 0.2 mm. per microgram. Observers usually have difficulty in duplicating readings of the same solution more closely than within 0.2 mm. Moreover, the No. 42 violet filter required for the evaluation of the color is at the part of the visual spectrum where the average analyst has most difficulty in duplicating readings. The extraction procedure is therefore probably not more accurate than within 2 micrograms over the range studied. Satisfactory percentage recoveries within a deviation of about 4 per cent or less can, therefore, be obtained only with quantities of selenium in excess of 50 micrograms. The procedure apparently lacks sensitivity for small quantities, although it is

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 23, 218 (1940).

probably satisfactory as a rapid one for the estimation of relatively large quantities.

(2) *Extraction of Selenium with Carbon Tetrachloride.*—A series of comparable extractions with carbon tetrachloride was made. The resultant color of selenium in the solvent is no more intense. In addition, the element is less soluble in carbon tetrachloride than in carbon disulfide, so that beyond 50 microgram quantities it is not completely dissolved. Since both the range and the accuracy are restricted, a colorimetric procedure based upon the extraction of selenium with carbon tetrachloride has probably no merit.

(3) *Extraction of Iodine with Carbon Tetrachloride.*—Studies were also made regarding the possibility of an accurate estimation of the iodine present in excess in the Norris-Fay titrimetric method by evaluating the violet color of the carbon tetrachloride extract. The plan was to add a fixed excess quantity of standard thiosulfate reagent to solutions containing 0–10 micrograms of selenium as selenious acid, then to add a fixed excess of standard iodine, and the excess was to be extracted with carbon tetrachloride and determined colorimetrically instead of by titration in the usual manner.

Accordingly, to prepare the standard iodine curve varying quantities of standard 0.0005 *N* iodine containing 5 grams of potassium iodide per liter were adjusted to a volume of 25 ml. with water and dilute hydrobromic acid. The solution was shaken with 5 ml. of carbon tetrachloride; the extract was drained through cotton into a 4 inch glass cell; and the resultant iodine color was evaluated in the photometer, a green No. 51 filter being used. The results are shown in Table 6.

TABLE 6.—Color density of *I* in *CCl<sub>4</sub>*

0.0005 <i>N</i> IODINE	<i>I<sub>2</sub></i>	<i>Se</i> EQUIVALENT	PHOTOMETER READINGS IN MM.			AVERAGE
			1	2	3	
ml.	micrograms	micrograms				
—	—	—	3.8	4.6	3.5	4.0
0.25	15.9	2.48	10.2	9.5	10.0	9.9
0.50	31.7	4.95	16.5	18.3	16.2	17.0
0.75	47.6	7.44	24.5	24.3	23.3	24.0
1.00	63.5	9.90	30.7	30.7	20.9	30.4
1.25	79.3	12.40	36.3	36.0	37.2	36.5

The photometer readings are not so reproducible as had been expected; 1.25 ml. of the iodine reagent or its stoichiometric equivalent of 12.4 micrograms of selenium contributes an increase of approximately 34 mm. scale divisions. One microgram of selenium should contribute approximately 3 mm. A deviation of 1 scale division from the average standard iodine curve would result in a variation of one-third microgram of selenium.

However, in actual practice, when determinations of added quantities of selenium as selenious acid were made, greater deviations than this were encountered. Attempts to remedy the discrepancies by increasing or diminishing the volumes of solution were of no avail. The average deviation was of the order of 1 microgram when 2–10 micrograms of the element were to be determined. This procedure therefore lacks reproducibility and accuracy in the range of selenium studied.

(4) *The Codeine Sulfate Method.*—Since the investigators cited in this paper had reported favorably on the colorimetric evaluation of selenium with the codeine sulfate reagent, the Associate Referee devoted some time to its study. The codeine-selenium complex varies from light green to deep blue, depending upon the quantity of the element present. The procedure has several inherent disagreeable features. Since water causes fading of the color complex, the reaction must be carried out in concentrated sulfuric acid. Consequently, the transfer of solution, making to volume, etc., must also be effected with the concentrated acid. Moreover, the complete color formation is not instantaneous. Gortner (*loc. cit.*) postulates that 7 hours' standing in the dark is imperative. Another investigator, *This Journal*, 22, 450 (1939), states that 2 hours is sufficient, but stipulates the need for iron in stabilizing the color, although Schmidt<sup>4</sup> supposedly demonstrated that iron is an interference in the reaction.

To determine the time required to develop the color, the transmission curves of two concentrations of the complex, that produced by 100 and 150 micrograms of selenium, were taken at hourly intervals. Selenium was added as selenious acid in concentrated sulfuric acid and diluted to 20 ml. volume with the acid; 5 drops of 3 per cent codeine sulfate were added from a buret, and the volumetric flask containing the selenium was shaken vigorously after the addition of each drop of reagent. Then the volume was adjusted to 25 ml. with concentrated acid. (This procedure was followed in obtaining all the data obtained on the codeine sulfate method presented in this paper.) The solutions were protected from light during the interval between readings. Charts 2 and 3\* illustrate the results. The character of the complex changed rapidly during the first hour of development for both concentrations, but after the second hour the color appeared to register fairly constant, especially in the region 560–600  $m\mu$ .

Transmission curves of three concentrations in duplicate after 7 hours' development are illustrated in Chart 4. The average per cent transmission for the quantities 150, 100, and 50 micrograms of selenium are 62.1, 71.8, and 83.1, respectively. While the concentrations of selenium are in the ratio 3:2:1, the corresponding densities are 2.6:1.8:1, indicating that the

<sup>4</sup> *Arch. Pharm.*, 252, 161 (1914).

\* The measurements shown in Charts 2 and 3 were made by R. W. Stewart of the U. S. Food and Drug Administration, Washington, D. C.

color complex does not follow Beer's law. (Densities were taken from transmission tables in Brode's Chemical Spectroscopy.)

The Associate Referee made further studies of the Gortner procedure by developing the color for 7 hours in the dark and evaluating the densities in the neutral wedge photometer, employing a 4 inch cell and yellow filter No. 58. The determinations were made in quadruplicate. The results are shown in Table 7.

TABLE 7.—Codeine-*Se* color density after 7 hours

Se	PHOTOMETER READING				AVERAGE	INTERVAL
	1	2	3	4		
micrograms			mm.		mm.	mm.
—	8.7	7.0	8.0	9.3	8.4	
5	14.7	17.0	16.0	14.5	15.5	7.1
10	21.6	21.8	22.0	20.8	21.5	6.0
20	32.2	31.5	32.0	31.5	31.8	10.3
30	42.8	41.0	44.5	44.0	43.1	11.3
40	54.3	53.0	55.0	55.0	54.8	11.7
50	60.6	63.5	66.0	67.0	64.3	9.5

The data (Table 7) indicate that the reaction is quite sensitive but lacks strict reproducibility; 1 mm. scale division corresponds to approximately 1 microgram of selenium. In the development of the 5 microgram standard one value varied from the average by 1.5 mm. or by approximately 1.5 micrograms, a variation of 30 per cent. Likewise, one 50 microgram standard varied by 3.7 mm. from the average, a deviation of approximately 7 per cent. Chart 5 illustrates the deviation of single determinations from the average.

The experiment was repeated, and the colors were evaluated in the photometer after 2 hours' development. The data (Table 8) indicate that for this period of development the color density is not so great as that produced in 7 hours but that, nevertheless, corresponding increments of selenium still contribute comparable differences in density. That is, increments of 10 micrograms of selenium contribute increases in density corresponding to approximately a 10 mm. photometer division.

Chart 6 illustrates the departure of individual results from the average. The variations are no more serious than those encountered for a 7 hour development. On the basis of the data presented the need for the longer period of standing does not seem to be justified. The greatest variation in the 5 microgram quantity developed for 2 hours is approximately 1 microgram, or 20 per cent; that of the 50 standard is also 1 microgram or 2 per cent.

Experiments also indicate that iron does not stabilize the codeine sulfate-selenium complex but rather that it is an interference. Ferrous sulfate,

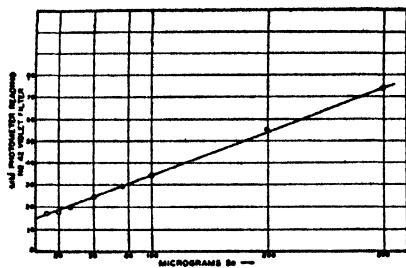


CHART 1

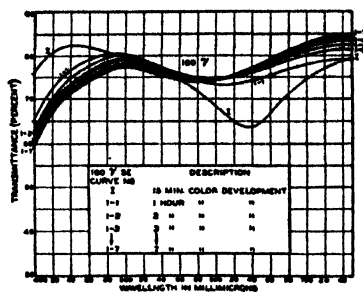


CHART 2

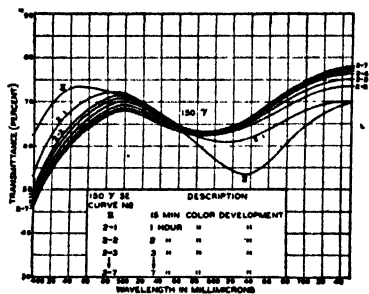


CHART 3

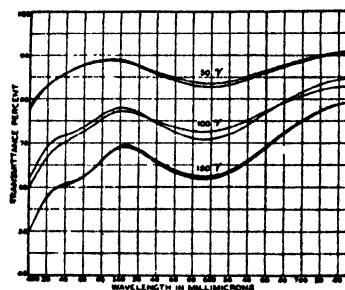


CHART 4

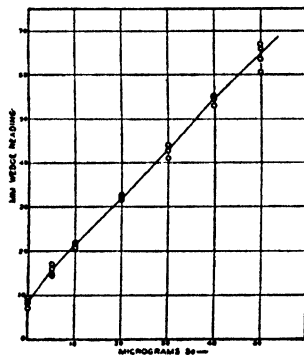


CHART 5

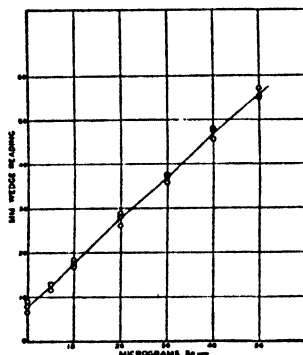


CHART 6

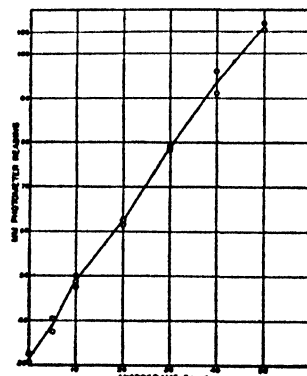
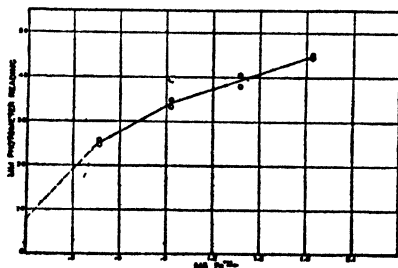


TABLE 8.—*Codeine-Se color density after 2 hours*

Se	PHOTOMETER READING			AVERAGE	INTERVAL
	1	2	3		
micrograms		mm.		mm.	mm.
—	6.5	9.0	8.0	7.8	—
5	13.0	11.3	12.8	12.4	4.6
10	18.6	17.0	17.6	17.7	5.3
20	28.0	26.0	29.0	27.7	10.0
30	37.4	35.8	37.0	36.7	9.0
40	47.5	45.5	48.0	47.0	10.3
50	57.0	55.0	55.3	55.8	8.8

when present in quantities even as small as 0.5 mg. as Fe, prevented the normal development of the color of 50 micrograms of selenium. Ferrous sulfate is a reducing agent. The reduction of selenite to selenium by this reagent is much faster than the reaction between codeine sulfate and selenite so that invariably the yellow precipitate of selenium is in evidence.

Iron present as ferric sulfate is also an interference. Codeine sulfate reacts with trivalent iron also to produce a blue color. The intensity of the color is roughly proportional to the amount of iron present, as Table 9 would indicate.

TABLE 9.—*Interference of iron*

Fe <sup>+++</sup>	PHOTOMETER READINGS (2 HOUR COLOR DEVELOPMENT)	
	micrograms	mm.
—	—	8.0
0.46	0.46	25.8
0.46	0.46	24.8
0.92	0.92	35.0
0.92	0.92	33.2
1.38	1.38	37.8
1.38	1.38	40.5
1.84	1.84	45.0
1.84	1.84	44.5

Chart 7 indicates that the 0.46 mg. of ferric iron would contribute an apparent selenium content of approximately 20 micrograms, while 1.84 mg. of the metal simulates the color of approximately 40 micrograms of selenium. It seems obvious, therefore, that if the codeine sulfate method is used in the determination of selenium in products containing iron that rigid precautions should be taken to keep the iron content of the sample and standards alike. The imposed condition requires standard curves for all dissimilar products and even perhaps for like samples unless the selenium is isolated by distillation.

Chart 8 and the data in Table 10 also indicate that ferric iron neither stabilizes the color during the normal 2 hour development nor causes the complex to follow Beer's law more strictly; approximately 1 mg. of iron as ferric sulfate was present.

TABLE 10.—Codeine-Se color density in presence of iron

Se	PHOTOMETER READINGS		AVERAGE	INTERVAL
	1	2		
micrograms		mm.	mm.	mm.
—	30.5	32.5	31.5	
5	40.5	37.5	39.0	7.5
10	47.5	50.0	48.7	9.7
20	61.5	62.5	62.0	13.3
30	79.0	78.5	78.7	16.7
40	91.0	96.0	93.5	14.8
50	107.0	105.5	106.2	12.7

Ferric iron seems to contribute larger increments of color density for the same quantity of selenium than does the reaction between codeine and selenium alone. The variations between like standards, however, are of the same order.

It would seem, therefore, that although the investigators have made progress with the codeine sulfate method, one that only recently was simply an identity test, it still does not yield the uniformly reliable results obtained by the present tentative method.

#### RECOMMENDATIONS\*

In view of the rather critical studies of the various selenium methods presented, it would seem that the tentative method, with refinements, yields consistently superior results and should be used for the present. However, it is recommended that further work be done on a possible refined iodine-iodate titrimetric procedure and also on available turbidimetric methods.

#### REPORT ON FUMIGATION RESIDUES IN FOODS

By W. O. WINKLER (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The work this year was confined to methods for the determination of hydrocyanic acid. Two partially outlined procedures given in the Associate Referee's report of 1938, *This Journal*, 22, 349 (1939), for the final determination of hydrocyanic acid, the phenolphthalin method and the

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 58 (1941).

thiocyanate method, were more thoroughly investigated. These determinations, based on the formation of highly colored compounds, lend themselves readily to photometric measurement and therefore were selected as giving promise for the detection of minute quantities of cyanide. Results of the work on both methods were satisfactory.

### THIOCYANATE METHOD

A change in the procedure outlined in the report two years ago was necessary in order to effect more complete removal of the sulfur, which caused some interference in the color development. The use of acetic acid for decomposing the sulfide used and separating the sulfur was found to be more satisfactory than that of mineral acid. The procedure is as follows:

#### PROCEDURE

Transfer the solution containing 3 ml. of a 15% solution of  $\text{Na}_2\text{S}$  crystals in which the HCN has been absorbed to a 250 ml. beaker with the aid of a little water from a wash bottle. Evaporate the solution to dryness on the steam bath and allow it to remain on the bath for 10 minutes after it becomes dry. Wash down the sides of the beaker with a few ml. of water and again evaporate the solution to dryness and allow it to remain on the steam bath a few minutes after the liquid is evaporated.

Take up the residue in 8 ml. of distilled water. Add 1 ml. of acetic acid (1+3) and after testing with litmus to insure an excess of acid, again evaporate to dryness on the steam bath. Remove the beaker from the bath and measure 5 ml. of water into it with a bulb pipet. Triturate the liquid with a rubber policeman and then filter the material through a heavy asbestos mat in a Gooch crucible, using a filtering bell jar. Receive the liquid in a 50 ml. volumetric flask. Wash the beaker with two more separate 5 ml. portions of water, measuring each portion. Finally, wash down the funnel with 2 ml. of water. (The filtrate should be clear or only faintly opalescent.) Add 3 ml. of  $\text{HNO}_3$  (1+1) and then add ethylene glycol monomethyl ether (methyl cellosolve) with occasional mixing to about 48 ml. and again mix the liquids (by inverting). Mark the flask with a glass marking pencil at about 48 ml. volume before using it.

Add 1 ml. of the reagent, a strong solution of  $\text{FeNH}_4(\text{SO}_4)_2$ , make to the mark with the ethylene glycol monomethyl ether, and again thoroughly mix by inverting the flask a number of times. (The same ferric solution should be used each time in samples and standards and it is necessary to measure accurately the water, acid, and ferric solution used.)

Allow the sample to stand 20 minutes and read in a neutral wedge photometer, Clifford type, *This Journal*, 19, 130 (1936), using a light filter centered at a wave length of 490  $\text{m}\mu$  in a 2-inch cell. Prepare a standard curve from the readings obtained by using graduated quantities (0.5–4 ml.) of standard 0.001 *N*  $\text{NH}_4\text{CNS}$  or  $\text{KCNS}$ , which have been made up with the same percentage of the liquids used in the sample. Plot micrograms of HCN as abscissas against photometer reading, as ordinates. The result is a straight line. In preparing the standard, adhere strictly to the quantity of aqueous solution used in the samples (total of 17 ml. of aqueous solution plus 3 ml. of  $\text{HNO}_3$  (1+1) made up to about 48 ml. with ethylene glycol monomethyl ether, reagent added, etc.).

Conduct a blank determination on the reagents by the procedure used on the samples. Read the quantity of HCN in the sample from the curve and subtract that



in the blank. For quantities in excess of 110 micrograms, use a smaller cell and prepare a new curve, using larger quantities of standard thiocyanate.

Some results by the method are given in Table 1.

TABLE 1.—*Recoveries of HCN (as KCN) by the production of thiocyanate and photometric determination*

SAMPLE NO.	HCN ADDED	HCN FOUND*	ERROR
	micrograms	micrograms	per cent
1	13.5	13.3	1.4
2	27.0	26.0	4.0
3	54.0	52.6	2.6
4	54.0	53.6	0.8
5	81.0	80.7	0.4

\* After subtraction of blank. HCN in reagents, Samples 1 to 4, inclusive, 12.4 micrograms; in Sample 5, 8.3 micrograms.

Results by the method are highly satisfactory, the only objection being the high blank obtained on the reagents, which appears to be contributed almost entirely by the sodium sulfide used since all the other reagents were used in the standards and gave no such blank. Plotted results on samples containing known added quantities of KCN gave a curve as straight as that obtained on the standards and parallel to it. Various samples of sodium sulfide should be tested to determine whether the blank can be lowered.

#### PHENOLPHTHALIN METHOD

The phenolphthalin method possesses several advantages over the thiocyanate method, but it also has its disadvantages. It has the advantage of being much more rapid and more easily applied and of giving a low blank determination. The reaction, while even more delicate than the thiocyanate reaction, is more erratic, and the points do not fall so well on the curve. It has the further disadvantage of not being specific for cyanide.

It was stated in the previous report that the curve produced by plotting the amounts of hydrocyanic acid against photometric readings appeared to be parabolic. The work this year indicates that this is true above 80–90 micrograms of hydrocyanic acid, but up to this amount the curve appears to be practically a straight line. The procedure used is as follows:

Transfer the basic solution (containing 2 ml. of normal NaOH or 1 ml. of 10% NaOH), in which the HCN was absorbed from the separation by aeration, to a 50 ml. volumetric flask, having marked the flask at about 48 ml. volume with a glass-marking pencil before using it. Use water from a wash bottle to complete the transfer. Add 15 ml. of 95% alcohol measured with a bulb pipet. Make up to about the 48 ml. mark with water and mix. Now add 1 ml. of phenolphthalin reagent, *This Journal*, 22, 352 (1939), to the sample, make to the 50 ml. mark, and mix by inverting a number of times. Allow the sample to stand for 20 minutes. Make a photometric reading on the solution in a neutral wedge photometer (Clifford type), using

a 2-inch cell and a light filter centered at 560 m $\mu$ . Prepare a standard curve by reading the color produced by quantities of 0.001 *N* KCN graduated from 0.3 to 3 or 4 ml. made to the same volume and containing the same volume of alcohol, base, water, and reagents as the sample. Obtain the HCN from the curve.

Some recoveries by the phenolphthalin method are given in Table 2.

TABLE 2.—*Recoveries of HCN from samples containing known quantities by the phenolphthalin photometric method*

HCN ADDED	HCN FOUND	ERROR
<i>micrograms</i>	<i>micrograms</i>	<i>per cent</i>
10.8	12.1	12
21.6	21.3	1.5
40.5	36.5	9.8
54.0	54.5	1
8.1	8.4	4
18.9	16.5	12
40.5	38.5	5
24.3	26.0	7

The results (Table 2) are quite satisfactory. The method is capable of detecting minute quantities of hydrocyanic acid with fairly good accuracy, and it should prove to be of value in food work.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That collaborative work be done on the two procedures given in this report.
- (2) That collaborative work be done on the aeration procedure given in the last report, *This Journal*, 22, 350 (1939).

### REPORT ON FRUITS AND FRUIT PRODUCTS

By B. G. HARTMANN (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

No formal reports were received on the subjects of sodium and chlorides, polariscopic methods, gravimetric phosphoric acid, and electrometric titration. Comprehensive reports on the remaining assignments—potassium oxide, colorimetric phosphoric acid, and fruit acids—were submitted

The sodium and chloride project is new. The chapter on Fruits and Fruit Products, *Methods of Analysis*, A.O.A.C., 1940, 335, does not list procedures for these two components. The assignment was made to determine the applicability of the existing methods to fruit products. The asso-

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 58 (1941).

ciate referee has done some work on the subject, but he considers that the results obtained do not justify a formal report. Further study is recommended.

In regard to the study of polariscopic methods for the determination of sugars, it was recommended last year that lead acetate be used on the alcoholic filtrate obtained in the pectin precipitation. It is suggested that mercury salts be also tried for the removal of optically active interferences. It is believed that mercury salts will more effectively remove the pigments of highly colored fruit products than will lead acetate.

In an informal communication, H. Shuman, Associate Referee on Gravimetric Phosphoric Acid, discusses plans for next year's work. He suggests examination of fruit products by the gravimetric, volumetric, and colorimetric methods. This plan has the full approval of the Referee, since it would furnish the data necessary to compare the effectiveness of the three procedures that are now available for the determination of phosphoric acid.

The assignment of electrometric titration has been carried by the Association for several years. Its purpose was to develop means for determining the acid components of fruit products. In his report of last year, the associate referee showed experimentally that it is possible to measure accurately the components of a mixture of two dibasic acids in pure solution from a titration curve. The titer of an acid solution measures free acids only; ordinarily it can not be used to arrive at the total acid content of a fruit product. It is therefore apparent that before electrometric titration can be of service in acid analysis, it will be necessary to formulate procedures for obtaining the acids free from interfering substances.

It is recommended that study of electrometric titration be discontinued until dependable procedures for the isolation of the free acids have been devised.

The study of the colorimetric method for the determination of  $P_2O_5$  in fruit products has now progressed to the point where it can be offered for approval as an official method, first action. The data recorded in support of this recommendation require no elucidation; the excellent results by the collaborators justify the recommendation.

The associate referee suggests a comparative study of the colorimetric and gravimetric procedures. While such a study is desirable, the Referee wishes to call attention to a similar suggestion by the associate referee on the gravimetric method.

A paper by the associate referee on the determination of potassium oxide in fruit products by the Wilcox cobaltinitrite method has been submitted for publication in *This Journal* (see p. 454). In this paper a large number of analyses by the platinate and cobalt methods on a large number of samples of fruit products is presented. The associate referee conducted a collaborative study of a modified Wilcox procedure based

upon the satisfactory behavior of the cobalt method. The results obtained by the collaborators are satisfactory on the whole; but on the high potassium oxide concentrations they are decidedly inconsistent compared with results by the platinate method. It is evident that the procedure requires further attention before it can be offered for adoption as a tentative method.

The Referee concurs in the recommendation that the chloroplatinate method be further studied with regard to clarification of details in the method. Presumably the recommendation is made with the view to introducing into the text amplifications that would clarify some of the provisions of the otherwise satisfactory method.

The report on fruit acids requires no discussion. From the work that has been done on the determination of citric acid it is believed that the proposed method is now in shape for collaborative study.

No work has been done on the determination of lactic acid. The colorimetric method described in *This Journal*, 20, 605 (1937), appears to be acceptable, and it is to be submitted to collaborative study.

Regarding methods for isocitric, succinic, and malic acids, the associate referee points out that sufficient ground work has now been done to warrant the formulating of procedures. It is realized that it will not be possible to do much collaborative work, but it is hoped that a procedure for isocitric acid will be offered for study.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That further study of the subject of sodium and chlorides and of polariscopic methods be made.
- (2) That study of electrometric titration be discontinued.
- (3) That the colorimetric  $P_2O_5$  procedure, *Methods of Analysis*, A.O. A.C., 1940, 347, 39, be adopted as official (first action).
- (4) That further study of the cobaltinitrite procedure for  $K_2O$  be made.
- (5) That collaborative study of the proposed citric acid procedure and of the lactic acid procedure, *This Journal*, 20, 605 (1937), be made.
- (6) That further study of methods for the determination of fruit acids be made.

No report on the electrometric titration of acids was given by the associate referee.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 64 (1941).

## REPORT ON FRUIT ACIDS

By B. G. HARTMANN (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Work this year was devoted to an investigation involving the readjustment of the official procedures for the determination of fruit acids, as it was recognized that the present methods are too laborious and time consuming to be of general service in food-control work. Convinced that the procedures could be greatly simplified, the Associate Referee subjected the various steps relating to the isolation and ultimate determination of the acids to a critical study.

It is not the intention at this time to present a detailed report of all the work that has been done, but it may be stated that as a result of the investigation it is now possible to determine the common fruit acids in materially less time than has heretofore been possible. Preliminary work on mixtures of tartaric, citric, isocitric, laevomalic, and inactive malic acids in pure solution can be determined in a single sample in less than two days of working time. Although very gratifying results on pure solutions have been obtained and in a few instances on fruit products themselves, some of the steps need further attention before a completed scheme can be offered for collaborative trial.

In the course of the work a procedure was developed for isocitric acid, for which, at this time, no method is offered in the literature. The lead salts of the isolated polybasic acids, after the removal of tartaric acid, are treated with acetic acid and alcohol, in which vehicles the citrates, normal and iso modification, are insoluble. Isocitric acid is then obtained as the difference between the total citric acid determined by titration and the normal citric acid determined as pentabromacetone.

A simple and reliable method for succinic acid has also been devised; it is based on the well-known behavior of the acid toward strong oxidants.

The procedure for the titrimetric determination of isolated acids, described in *This Journal*, 22, 357 (1939), was readjusted and made the basis of group determinations for citric and malic acid.

In a previous report, *Ibid.*, 360, it was shown that slow oxidation of the brominated citric acid solution yields appreciably more pentabromacetone than does rapid oxidation. The determination has been rewritten to admit of this refinement.

It has been pointed out in former reports that tannin interferes with the polariscopic determination of malic acid, but it was found that treatment of the malic-uranium complex with sensitized charcoal obviates the trouble.

These are some of the achievements of this investigation, but their introduction into a workable scheme of acid analysis will require much more time.

A detailed report of the determination of citric acid in fruits and fruit products is presented in this report. The method, which is an adaption of the official procedure in principle, embodies certain innovations that not only shorten the time of operation but produce more accurate results in lower acid concentrations.

Of the several methods that have been recommended for the determination of citric acid, the pentabromacetone procedure is now generally accepted as the simplest and most reliable. In its operation complicated apparatus is not necessary, an advantage that makes the method particularly suitable for food-control work. Under controlled conditions the "penta" reaction is sensitive to an astounding degree, as by its means 1 mg. of acid in pure solution can be detected with certainty in 100 ml. reaction mixture or 1 part in 100,000.

Much has been written regarding the care required for the conversion into penta. High temperatures and agitation during the bromination-oxidation period are reported by Schulze<sup>1</sup> as being definitely harmful to the reaction. Over oxidation of penta is stressed as one of the influences adversely affecting the reaction, and one investigator, in order to minimize the breaking down of the compound through overoxidation, recommends the introduction of ammonium sulfate.

The results obtained in this investigation show conclusively that the precautions cited are unnecessary. It would seem that irregular results are attributable not to any fault of the reaction itself, but more directly to the manner of collecting and measuring small quantities of pentabromacetone.

Kometiani<sup>2</sup> recommends the iodometric titration of the compound. The alcoholic solution of the penta is treated with potassium iodide, and the liberated iodine is titrated with thiosulfate solution in the usual manner. In this laboratory the procedure did not prove entirely satisfactory. No trouble was experienced with solutions of pure citric acid, but in admixture with large quantities of other fruit acids the results were low and erratic. In the 1939 edition of his "Laboratoriumsbuch für den Lebensmittel Chemiker" Beythien cites the Reichard procedure,<sup>3</sup> in which the penta is weighed as in the method now exclusively used by the food chemist. Beythien merely mentions the iodometric determination, from which it may be inferred that the procedure has not found favor in German laboratories. In the procedure here proposed the penta is measured gravimetrically.

Two methods for drying penta are available: aeration and drying over sulfuric acid. From the standpoint of accuracy either procedure is suitable; aeration is preferred because it is less time-consuming.

As has been repeatedly pointed out in former reports, the lead salts of

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<sup>1</sup> *Z. Untersuch. Lebensm.*, **67**, 605 (1934).

<sup>2</sup> *Z. anal. chem.*, **86**, 359 (1931)

<sup>3</sup> *Z. Untersuch. Lebensm.*, **72**, 50 (1936).

polybasic acids are appreciably soluble in lead acetate. The addition of the salt can be more closely controlled by calculating the quantity necessary for the precipitation of the acids from the titer of the alcoholic solution obtained in the pectin precipitation.

The main change in the method was made in the conversion of citric acid into penta, since, as has been pointed out, slow oxidation permits of a strictly quantitative return. It is of interest to note that Kunz<sup>4</sup> recommends gradual oxidation of the brominated acid solution.

In the calculation of citric acid no solubility correction is made; the weight of penta multiplied by the theoretical conversion factor 0.424 gives anhydrous citric acid.

For the collection of penta small Knorr tubes are used. Tubes of this type have the advantage over the Gooch crucible in that they require only a small quantity of asbestos, thus facilitating washing and drying operations. Also the tubes, because of their small openings, can be more easily fitted into the drying train. It was found that it is not necessary to cool the air used in aeration.

The method follows:

#### CITRIC ACID

##### 1

##### PREPARATION OF SAMPLE

In the case of a jam or preserve prepare a sample solution as directed in *Methods of Analysis*, A.O.A.C., 1940, 335, 2(b<sub>1</sub>). Pipet 200 ml. of sample solution into a 600 ml. beaker and boil down to about 35 ml. While still hot add 2 N H<sub>2</sub>SO<sub>4</sub>, mix thoroughly, and allow to stand a few minutes. Pour into a 250 ml. volumetric flask and rinse beaker with 15 ml. of hot water. Rinse beaker with 95% alcohol, cool, add alcohol to mark, and shake a few minutes. Adjust with alcohol to 20°C., pour onto a large funnel (about 4") lined with a layer of absorbent cotton, and collect about 220 ml. of filtrate. (Toward the end filtration is slow, but by gathering the ends of the cotton and squeezing the incased residue the desired quantity of filtrate is obtained.)

In the case of jelly, weigh 30 grams of sample into a 250 ml. beaker, add 25 ml. of water, and boil down to 35 ml. Proceed as directed for jams.

In the case of fruit juice transfer 20 grams of sample with 30 ml. of water to a 250 ml. volumetric flask, add 2 N H<sub>2</sub>SO<sub>4</sub>, and place on steam bath for 5 minutes. Cool, and proceed as directed for jams.

##### 2

##### ISOLATION OF POLYBASIC ACIDS

Determine titer, "t," of 10 ml. of the filtrate in terms of 0.1 N alkali. Into a centrifuge bottle of about 250 ml. capacity, pipet 200 ml. of filtrate and add "0.6 t" gram of powdered lead acetate. (The value "0.6t" is derived from  $0.03t \times (200/10)$ , in which 0.03 represents grams Pb(ac)<sub>2</sub> · 3H<sub>2</sub>O. The quantity of lead salt is excessive by approximately 50% and is necessary to take care of combined acids.) Stopper bottle, shake vigorously 5 minutes, and let stand 10 minutes, shaking frequently. Centrifuge at high speed, carefully decant clear liquor, and discard. Test liquor with a small quantity of saturated lead solution and if necessary add more solution and centrifuge again. To the lead salts add 50 ml. of 80% alcohol (80 ml. 95% alcohol + 20 ml. water) and shake until salts are completely dispersed. Fill the bottle with

<sup>4</sup> Arch. Chem. Mikro., 7, 285 (1914).

the alcohol, shake thoroughly, centrifuge, and discard liquor. Repeat the last step. Disperse lead salts in 50 ml. of water, add 50 ml. of water, and saturate with  $H_2S$  gas. With water transfer solution to a 250 ml. volumetric flask, shake thoroughly, make to mark, mix, and pass through a large fluted paper, pouring back until bright. In a 400 ml. beaker boil down 200 ml. of filtrate to about 50 ml.

## 3

## DETERMINATION

Rinse solution into a tared 300 ml. glass-stoppered Erlenmeyer flask and boil down to 40 grams. Cool, add 2 grams of KBr and 5 ml. of  $H_2SO_4$  and warm to 45°–50°C. Let stand a few minutes and from a buret add immediately 25 ml. of 5%  $KMnO_4$  solution dropwise. After the  $KMnO_4$  has been added, swirl about 1 minute and place in refrigerator 30 minutes. Swirl about 1 minute and add  $FeSO_4$  solution (dissolve 200 grams of  $FeSO_4 \cdot 7H_2O$  in water, dilute to 500 ml., add 5 ml. of  $H_2SO_4$ , and filter). Add iron solution slowly with constant shaking until the mixture starts to clear up. Shake vigorously and continue addition of iron solution until the  $MnO_2$  is dissolved. Add a few ml. in excess, cool to 15°C., add 20 grams of powdered anhydrous  $Na_2SO_4$ , stopper flask, and shake 3 minutes. Collect the precipitate of penta on asbestos in a small Knorr tube. (The tube is the type used in the determination of fat in cacao products. It has a body about  $\frac{3}{4}$ " in diameter by  $1\frac{1}{2}$ ", and a stem  $1\frac{1}{2}$ "

TABLE 1.—*Citric acid in admixture with other acids in pure solution*

SOLUTION CONTAINED	CITRIC ACID RETURNED		SOLUTION CONTAINED	CITRIC ACID RETURNED		
mg.	mg.	per cent	mg.	mg.	per cent	
0.5 citric	Slight indication of penta		73.9 citric	74.4	100.5	
100 tartaric			25 tartaric	74.3 74.3		
				74.2		
1.0 citric	Strong indication of penta		98.5 citric	99.2	100.7	
100 tartaric			No tartaric	99.2 99.2		
2.0 citric	1.7	80.0	98.5 citric	98.9 99.0	100.5	
	1.6					
100 tartaric	1.5 1.6		100 tartaric			
	1.6					
4.9 citric	4.4	89.8	197.0 citric	198.5 198.5	100.8	
	4.3					
100 tartaric	4.4 4.4		No tartaric			
9.9 citric	9.5	97.0	No citric	Opalescence No indication of penta		
	9.7 9.6					
90 tartaric.	9.5		100 tartaric			
24.6 citric	24.4	98.8	49.3 citric	48.8	99.0	
	24.3 24.3					
75 tartaric	24.3		50 malic			
49.3 citric	49.0	99.6	49.3 citric	49.1	99.6	
	49.0 49.1					
50 tartaric	49.2		50 succinic			



long. At its upper end it is provided with 2 small glass toes to prevent rolling while weighing.) Wash the flask with a portion of the filtrate and wash the tube with about 50 ml. of water cooled to 15° C.

Dry the penta over  $\text{H}_2\text{SO}_4$  in a vacuum desiccator overnight or by aeration. If aeration is used, wipe inside of tube with a small piece of filter paper and push paper down into tube. Place tube in drying train, aerate 10 minutes, and weigh. (The air is dried by drawing through  $\text{H}_2\text{SO}_4$  and soda lime. The assembly for drying is conveniently made up of 2 suction flasks containing  $\text{H}_2\text{SO}_4$  and a drying tower filled with soda lime.) Pass the air through the system by means of suction at a slow uniform rate. Aerate an additional 5 minutes and reweigh. If loss in weight exceeds a few tenths mg. continue aeration. Remove penta with alcohol and ether. Fill tube 3 times with each solvent. Aerate tube 5 minutes and reweigh. The difference between weights, times 0.424, equals grams anhydrous citric acid in aliquot.

The procedure was tried on solutions of pure acids and on fruit products. The results (Table 1) were obtained directly on the acid solutions. The acids were transferred to the Erlenmeyer flask, and the pentabrom-acetone was determined as directed in the method. The results in Table 2 were obtained by adhering strictly to the provisions laid down in the method.

TABLE 2.—*Added citric acid in fruit products*

PRODUCT	CITRIC ACID ADDED	CITRIC ACID DETERMINED	ADDED CITRIC ACID RECOVERED	
			mg.	per cent
Apple jelly	None	4.7 4.5 4.6	—	—
Apple jelly	4.9	9.3	4.7	95.9
Apple jelly	49.3	53.6	49.0	99.4
Apple jelly	98.5	101.4	96.8	98.3
Apple jelly	147.8	151.5	146.9	99.4
Concord Grape Juice	None	4.7 4.6 4.7	—	—
Concord Grape Juice	4.9	9.6	4.9	100.0
Concord Grape Juice	49.3	53.7	49.0	99.4
Concord Grape Juice	98.5	102.7	98.0	99.5
Concord Grape Juice	147.8	152.0	147.3	99.7

## DISCUSSION OF RESULTS

The results recorded in Table 1 show an appreciable loss of citric acid in the lower brackets. That the loss is not due to any fault of the reaction,

however, is evidenced by the fact that 0.5 mg. of citric acid can still be definitely detected. Apparently the loss is due to the slight solubility of penta in the 50 ml. of wash water. Attempts to demonstrate this by collecting the penta in Pregl tubes and washing with 10 instead of 30 ml. of water were not entirely successful. Filtrations were slow, and some difficulty was encountered in the transfer of the precipitate with the 10 ml. of water. However, duplicate determinations on a solution containing 1 mg. of citric acid and 100 mg. of tartaric acid gave returns of 40 and 60 per cent.

### SUMMARY

The pentabromacetone reaction for the determination of citric acid under controlled conditions is sensitive to 1 part in 100,000; 1 mg. of citric acid in admixture with 100 mg. of tartaric acid returned about 50 per cent of the acid.

The proposed procedure is easy of manipulation and requires no particular experience. In the calculation no solubility correction is applied; the weight of pentabromacetone multiplied by the theoretical conversion factor 0.424 gives anhydrous citric acid. The time required for the determination of citric acid from the point where the alcoholic filtrate from the pectin precipitation has been obtained is about 3 hours.

It is recommended that the proposed method be submitted to collaborative trial during the coming year.

## REPORT ON POTASSIUM OXIDE IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS

By C. A. Wood (U. S. Food and Drug Administration, New  
York, N. Y.), *Associate Referee*

The Wilcox cobaltinitrite method for potassium<sup>1</sup> is more rapid and requires less manipulation than does the official chloroplatinate procedure. The writer has made slight modifications of this method in applying it to fruit products. Since the Wilcox cobaltinitrite technic has been found useful in this field, it was submitted to collaborative study.

Four samples were prepared as follows:

- A. Sample solution of cherry fruit plus 0.1% salicylic acid as a preservative.
- B. Sample solution of this fruit plus 360 mg. of  $K_2O$  per 100 gram of sample.
- C. Pure sugar plus 10 mg. of  $K_2O$  per 100 grams of sugar. The ash content of the sugar was 1 mg./100 grams and the  $K_2O$  blank by the chloroplatinate method 0.5 mg. per 100 grams.
- D. A solution of a mixture of fruit ashes, apricot, cherry, strawberry, and raspberry.

Collaborators were instructed to make analyses by the cobaltinitrite,

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<sup>1</sup> *Ind. Eng. Chem.*, 9, 136-138 (1937).

gravimetric, and tentative chloroplatinate methods. Provision was made to correct for any insoluble residue in the potassium chloroplatinate precipitate by weighing the Gooch with the precipitate and again after washing it out with hot water. Aliquots taken for the cobaltinitrite precipitation were adjusted between 2.4 and 18 mg. of potassium oxide, the range of this estimation in 15 ml. volume. The actual weights of sample in aliquots taken are indicated in the table of collaborative results. Findings are given in mg. of potassium oxide per 100 grams of sample.

*Collaborative results*

ALIQUT USED FOR PRECIPITATION (GRAMS)	SAMPLE A		SAMPLE B		SAMPLE C		SAMPLE D	
	15	6	7.5	3	30	24	15	10
	CHLORO- PLATI- NATE	COBAL- TINI- TRITE	CHLORO- PLATI- NATE	COBAL- TINI- TRITE	CHLORO- PLATI- NATE	COBAL- TINI- TRITE	CHLORO- PLATI- NATE	COBAL- TINI- TRITE
San Francisco	204	205	561	527	11.2	*	155	153
H. W. Gerritz	203	199	567	553	11.5		156	151
St. Louis								
S. D. Fine	189.3	203.6	544	557	8.7	8.9	151	157
J. T. Field	195.3	201.5	541	536	9.5	*	153	156
Chicago	193.6	181.8	550.8	511.5	10.3	9.2	148.0	138.5
R. T. Stanley	195.6	183.2	549.7	499.8	9.8	9.5	143.7	140.4
Washington								
A. Wolf	195.6	195.9	†	†	9.3	10.0	146.4	141.6
R. A. Osborn	206.0	191.6	†	512.2	10.9	10.7	157	142.9
	202.0	186.6	†	501.9	11.2	11.1	157	145.0
New York								
C. A. Wood	198.4	202.0	554.2	543.8	9.3	11.0	150.6	151.8
	199.7	199.9	553.6	539.7	9.4	11.0	153.0	152.0
J. L. Hogan								151.0
F. B. Jones		201		541.3				
				558.7				
Minimum	189.3	182	541	500	8.7	8.9	143.7	138.5
Average	198.4	195.9	553	532	10.1	10.2	151.9	148.4
Maximum	206.0	205	567	559	11.5	11.1	157	157

\* Results omitted; aliquot taken below range for cobaltinitrite method.

† Results 450, 465, 470, chloroplatinate, 457 cobaltinitrite, omitted; solution prepared at different time than others and some question as to concentration.

### DISCUSSION OF ANALYTICAL RESULTS

The data obtained at the Chicago Station on the cobaltinitrite method are rather consistently near or at the minima. In this laboratory the reagent and aliquot for precipitation were not cooled to 20° C. before being

mixed. Although the directions to collaborators did not indicate this precaution, which may be material if the surrounding temperature is high, it has since been included in a contributed paper (see p. 454).

For small quantities of the metal, as in Solution C, the average recovery of potassium was better by the cobaltinitrite than by the chloroplatinate procedure. For unusually large quantities, which are encountered very occasionally, the average recovery by the official method is better.\* The average by both methods for A was 197 mg., and to this 360 mg. had been added. The theoretical amount present, therefore, was 557 mg. The average chloroplatinate recovery was approximately 99 per cent of this amount, and the average cobaltinitrite recovery was approximately 96 per cent. The cobaltinitrite average figures are a little lower than those by the official method for Samples A and D; for Sample C, which contained 10.5 mg., they are a little higher.

Collaborators agree that the cobaltinitrite method is more rapid and requires less manipulation than does the chloroplatinate method.

#### RECOMMENDATIONS†

It is recommended—

(1) That the modified Wilcox cobaltinitrite method be adopted as tentative, and that it be further studied with reference to recoveries of large quantities of potassium oxide.

(2) That further collaborative work be done leading to the application of the method to larger quantities of potassium in the aliquot taken for analysis.

(3) That the tentative chloroplatinate method be further studied in regard to clarification of details.

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No report on polariscopic methods for jams, jellies, and preserves was given by the associate referee.

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No report on gravimetric methods for the determination of  $P_2O_5$  in jams, jellies, and other fruit products was given by the associate referee.

#### REPORT ON $P_2O_5$ IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS (COLORIMETRIC METHOD)

By HAROLD W. GERRITZ (U. S. Food and Drug Administration,  
San Francisco, Calif.), *Associate Referee*

During the year a further study was made of the colorimetric method adopted as tentative at the last meeting.

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\* Better cobaltinitrite recoveries can probably be obtained when larger volumes are used for the precipitation. See data in contributed paper.

† For report of Subcommittee D and action by the Association, see *This Journal*, 24, 64 (1941)

Samples were sent to nine collaborators. The samples consisted of (No. 1) a cherry preserve, (No. 2) an apricot preserve (both prepared as directed in *Methods of Analysis*, A.O.A.C., 1935, 319, paragraph C) and (No. 3) a commercial grape juice (diluted 500 grams to a liter).

The samples were preserved with toluene. The collaborators were requested to take aliquots from below the toluene layer, and to digest and analyze 50 ml. aliquots of Samples 1 and 2 and 20 ml. aliquots of Sample 3 in duplicate, taking 20/100 aliquots for color development and following the method as described in *This Journal*, 23, 321 (1940).

Last year Alfend and Field obtained results on a grape jelly that indicated a possibility of loss of  $P_2O_5$  due to excessive charring and/or fuming during digestion. To further study this effect, the collaborators were requested to carry out the following experiment, which was expected to show the extent of such loss.

To a third 20 ml. portion of Sample 3 in a Kjeldahl flask, add 10 grams of phosphate-free sugar. Analyze according to the regular procedure with the following exception: after the initial addition of sulfuric and nitric acids, digest to such a char that  $SO_2$  fumes just begin to rise in the neck of the flask, then add more  $HNO_3$  and continue the digestion, repeating the charring and addition of the acid until a clear solution is obtained, then fume for an additional 15 minutes. Determine the approximate amount of  $H_2SO_4$  lost during digestion.

Collaborators were requested to report results in terms of milligrams of  $P_2O_5$  per 100 grams of original sample. Results are given in Table 1.

From personal observation of more than 500 determinations on various food products by ten analysts engaged in fruit products analysis at San Francisco Station, the Associate Referee believes that the results given in Table 1 are fairly representative of the accuracy of the method.

The standard deviations were calculated by treating each result as a separate observation with the exceptions indicated in the footnotes. In a similar manner, a standard deviation of 0.59 mg./100 grams was calculated from the entire group of 54 results. One result on Sample 1 (25.6 mg./100 grams) falls beyond a 100 to 1 probability ( $2.58 \times 0.59$ ) from the average of that sample and, therefore, logically might be ignored in judging the accuracy of the method.

The data in Column 4, obtained under conditions of excessive charring and fuming during digestion, fail to confirm previous indications of significant loss of  $P_2O_5$  due to such conditions. However, the results are more erratic and the Associate Referee believes that the analyst is well advised to exercise reasonable precautions.

#### HETEROGENEOUS SAMPLES

Results reported in Table 1 were obtained on water solutions, and therefore are not subject to sampling error. Since, however, the method is readily adaptable to the analysis of heterogeneous samples such as berry

TABLE 1.—*Collaborative results*  
(P<sub>2</sub>O<sub>5</sub>, mg./100 grams original sample)

ANALYST	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 3 SUGAR ADDED	% H <sub>2</sub> SO <sub>4</sub> LOST
Leon A. Salinger San Francisco	24.2 24.2	24.2 24.2	26.3 26.2	25.8	—
Harry Bois San Francisco	23.9 23.9	24.6 24.2	26.3 26.5	26.3	28
Sidney Kahan New Orleans	23.8 23.6	24.5 24.6	26.4 26.1	26.3	28
Robert O. Stanley Chicago	22.2}* 22.2} 22.8}* 22.8}	23.3}* 23.3} 23.1}* 22.9}	26.1}* 25.5} 26.2}* 26.1}	25.9 26.1	27
Harley G. Underwood Chicago	23.1}* 23.1} 22.9}* 23.0}	22.9}* 23.2} 22.9}* 22.9}	25.7}* 26.0} 26.0}* 25.9}	25.8 25.9	36
J. W. Sanders, Jr. Atlanta	24.0 24.1 24.0†	24.0 24.3 24.3†	26.4 26.2 26.3†	26.7	—
W. C. Woodfin Atlanta	22.9 23.0	23.1 23.1	25.4 25.2	24.9	—
C. H. Badger Washington, D. C.	25.6 24.2	24.5 23.4	25.9 25.0	25.8	29
R. A. Osborn Washington, D. C.	23.5 23.7	23.3 23.7	25.7 25.5	26.7	66
Average	23.6	23.8	26.0	26.0	
Standard deviation	0.74	0.61	0.37		

\* Duplicate color development on same digest averaged in calculating standard deviation

† Third result omitted from calculation of standard deviation in order to avoid unduly weighting this analyst's results.

products containing relatively large seeds, it was deemed desirable to conduct a collaborative study that would indicate the order of magnitude of sampling error for such products in order to establish a minimum practical charge.

A sample of Boysenberries was prepared by passing approximately 2 gallons of fresh berries through a Hobart grinder twice (using plate with holes 2/16 inch in diameter) and mixed with the Hobart mixing attachment. About  $\frac{3}{4}$  pint in a pint Mason jar was handed to each of five analysts

at the San Francisco Station with the following request: "Weigh out accurately and transfer to Kjeldahl flasks approximately 5, 10, 15, 20, and 40 gram portions. Determine  $P_2O_5$  according to the colorimetric method as described in *This Journal*, *loc. cit.*, using 5 ml. of  $H_2SO_4$  for the digestion of a 5 gram portion, and 10 ml. for the larger portions. Develop the color on each digest, and later again develop the color on each digest, reporting both results on each of the digests. Each analyst should develop color at two different times."

Results are presented in Table 2.

TABLE 2.—Results of using heterogeneous samples  
( $P_2O_5$ , mg./100 gram sample)

ANALYST	5 g. CHG.	10 g. CHG.	15 g. CHG.	20 g. CHG.	40 g. CHG.	AVER- AGE	SAME DIGEST FOR ALL ANALYSTS
H. M. Bollinger	55.9 56.1	59.8 59.3	57.4 57.3	56.5 57.1	58.0 58.1	57.6	58.0 58.1
D. W. Williams	58.9 58.9	56.9 57.1	57.9 57.6	57.0 56.9	57.9 57.7	57.7	59.0 58.9
D. A. Holaday	56.4 56.0	56.1 56.2	57.5 57.2	55.7 57.4	58.4 58.5	56.9	58.4 58.2
D. A. Ballard	58.8 58.9	58.1 57.8	59.3 59.3	57.7 57.4	56.3 56.5	58.0	57.8 59.0
L. A. Salinger	57.7 57.3	60.2 59.6	58.9 58.6	58.8 58.2	58.3 57.7	58.5	58.6 58.1
Average	57.5	58.1	58.1	57.3	57.7		

Data presented in Table 2 indicate that while there is a sampling error, its magnitude on charges of 5 grams or more bears no definite relation to size of charge. At this laboratory, a 15 gram charge is preferred.

#### SUMMARY

Collaborative data are presented to show limits of error of the colorimetric method for  $P_2O_5$ , both when applied to homogeneous samples such as water solutions of fruit products and to heterogeneous samples, such as ground Boysen berries.

Previous indications of loss of  $P_2O_5$  due to excessive charring and fuming during digestion were not definitely confirmed.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the colorimetric method for the determination of  $P_2O_5$  in

\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 64 (1941).

fruit and fruit products, adopted as tentative last year, be made official, first action.

(2) That further collaborative work be done next year on a comparative study of the A.O.A.C. colorimetric and magnesium pyrophosphate gravimetric methods for the determination of  $P_2O_5$ .

## REPORT ON SODIUM AND CHLORIDES IN FRUIT AND FRUIT PRODUCTS

By R. S. PRUITT (U. S. Food and Drug Administration,  
Cincinnati, Ohio), *Associate Referee*

### CHLORIDES

The significance of the ash in determining the amount of fruit used in fabricating fruit products is well recognized by food chemists. It is apparent that any added ash material, other than that from the fruit, must be corrected before the ash figure assumes its importance. It has been reported that partially refined invert sugar sirup, which may sometimes be used in jams and jellies, often has an ash content of approximately 0.3 per cent, which ash is largely sodium chloride.

The studies that were made during the past year were designed primarily to find or formulate a method that could be used to accurately determine such added chlorides. Three methods for chlorides were investigated, all having at some time received official action by the Association.

Method 1 is essentially the one given in *Methods of Analysis, A.O.A.C.*, 1935, 131, 34 and 37. The changes are as follow: 20 gram samples were used with 20 ml. of 10 per cent sodium carbonate as a fixative; twentieth normal solutions of silver nitrate and potassium thiocyanate were used as standard solutions; and 15 ml. of nitric acid (1+3) was used in place of the 1+4 dilution.

Method 2 is essentially the one published, *Ibid.*, 38 and 39. Powdered potassium permanganate was used instead of the solution. No potassium permanganate was added at the beginning, and from 6-8 grams was added at the end. After digestion of the filtered precipitate with copper sulfate and potassium sulfate and sulfuric acid, the solution was diluted to 200 ml. and boiled for 10 minutes to get rid of any sulfurous acid that might be present.

Method 3 is a modified open Carius method. The 20 gram sample, to which was added a measured excess of standard silver nitrate solution, was digested in a 500 ml. Erlenmeyer flask with 100 ml. of nitric acid on a steam bath for 2 hours. At the end of the digestion, 6-8 grams of powdered potassium permanganate was added slowly. The solution was diluted with



100 ml. of water, brought to a boil, and filtered. The excess of silver nitrate was then titrated.

The limited amount of work done so far would indicate that Method 3 has the greatest possibilities. All the work was done at the Cincinnati Laboratory by Messrs. McNall and Spruiell, and none of the methods has been submitted to collaboration.

*Results obtained by McNall and Spruiell*

SAMPLE*	Cl ADDED	Cl RECOVERED BY THE FOLLOWING METHODS—					
		I	II	III	I	II	III
	mg.		mg.			per cent	
Plum Preserves	None	None	None	None	—	—	—
	None	None	None	None	—	—	—
	9.94	7.94	9.04	10.11	79.88	90.95	101.7
	9.94	8.12	9.34	10.28	81.69	93.96	103.4
Cherry Preserves	None	None	—	None	—	—	—
	None	None	—	None	—	—	—
	9.94	7.26	—	9.43	73.04		94.9
	9.94	7.97	—	9.35	80.18		94.1
Black Raspberry Preserves	None	1.44	.27	.74		—	—
	None	1.13	.28	.76		—	—
		1.10					
	9.94	9.41	6.17	9.05	94.66	62.07	91.05
	9.94	9.49	5.92	9.02	95.47	59.56	90.74
		9.36			94.16		
Apricot Preserves	None	None		None			
	None	None		None			
	4.97	3.36			67.6		
	14.91	12.21			81.9		
	9.94	7.56		9.03	76.1		90.9
	9.94	7.75		8.94	77.9		89.9
	9.94			9.24			92.9
	9.94			9.52			95.8

\* All samples were prepared from authentic fruit.

### SODIUM

Some work was done on the determination of sodium in fruits and fruit products, but not enough to report further than that sodium is present in these products in very small quantities.

## REPORT ON VITAMINS

By E. M. NELSON (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

Vitamin A.—The recommendations of the associate referee that study of the method presented be continued is approved.

Vitamin B<sub>1</sub>.—It is recommended—

(1) That the method for the assay of vitamin B<sub>1</sub> proposed by the associate referee be adopted as tentative, and that collaborative studies be conducted.

(2) That the associate referee be requested to report on chemical methods for the determination of vitamin B<sub>1</sub> in flour and other cereal products at the next meeting.

Vitamin C.—No report.

Vitamin D.—Milk.—The recommendation of the associate referee that a uniform method for expressing degrees of healing be studied is approved.

Vitamin K.—It is recommended—

(1) That the method for vitamin K proposed by the associate referee be adopted tentatively, and that collaborative studies be conducted on the method.

(2) That the associate referee and referee be instructed to redraft the text so that it will conform with the style used in *Official and Tentative Methods of Analysis*.

Riboflavin.—It is recommended—

(1) That both the bacteriological and fluorometric methods presented by the associate referee be tentatively adopted for the assay of riboflavin in yeast and dried skim milk.

(2) That studies on the bacteriological and fluorometric methods be extended to liver meal, fish meal, alfalfa meal, and other materials.

(3) That a primary standard for riboflavin be studied in order to have a method for checking the purity of riboflavin solutions used as standards.

It is suggested that the associate referee be guided by the following remarks as to the extent to which the studies in Recommendation (3) be pursued. The Referee has been advised that official action has been taken to include riboflavin in the U. S. Pharmacopoeia. The Pharmacopoeial Convention will be requested to distribute a Reference Standard Riboflavin for use in assays. If such a standard becomes available the need for extensive investigation on a so-called primary standard may be minimized.

Vitamin D for poultry.—The Referee approves of the following recommendation of the Associate Referee: "Delete the words, "All chicks that weigh 100 grams or less and," from the text of the tentative method, *Methods of Analysis*, A.O.A.C., 1940, 372, line 21.

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## REPORT ON VITAMIN A

By J. B. WILKIE (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

At the meeting of the A.O.A.C. last year collaborative work was not recommended because a similar investigation was in progress under the direction of the Vitamin Advisory Board of the U. S. Pharmacopoeia. It has been one of the Associate Referee's duties and privileges to assist in this study and to make a preliminary analysis of the respective collaborative reports. Furthermore, authority has been granted through the U. S. Pharmacopoeia Vitamin Advisory Board to present a brief preliminary report of these results at this meeting. It is expected that a more complete report will be prepared for publication later through U. S. Pharmacopoeial channels.

To date reports have been received from ten collaborators using true spectrophotometric equipment. Reports from two other collaborators using only filter photometric equipment have also been received. Only the results obtained from the true spectrophotometers will be considered at this time.

Duplicate samples of three different oils, labeled U.S.P. 1, 2, and 3, respectively, were sent to each of the collaborators. Each sample of oil was treated by each collaborator in three different ways. One treatment consisted merely of diluting the oil with a 10 per cent cyclohexane 90 per cent absolute alcohol mixture for the spectrophotometric examination. Another procedure followed a definite saponification for obtaining the nonsaponifiable material for the spectrophotometric examination, *This Journal*, 23, 341 (1940). For the last procedure each collaborator was allowed to choose his saponification and extraction method.

A questionnaire covering details of apparatus and technic was filled out by each collaborator. Such details, while interesting and important, will not be covered in this report. The over-all performance of apparatus was standardized with potassium chromate as recommended at a previous A.O.A.C. meeting, *Ibid.*, 22, 465, 468 (1939).

The results of this study were statistically treated. As a unit of statistical comparison, the coefficient of variation was favored because it is comparatively easy to understand, involves comparatively simple calculations, and appears to possess significance leading to interesting conclusions. Its expression is entirely analogous to the expression of per cent; that is, zero indicates no spread of results, a small number as 2 or 3 indicates a small spread of results, and 100 indicates a complete spread of results.

Standard deviation appears to be the generally accepted measure for scatter, spread of results, or dispersion, but it must be divided by the average to obtain an absolute measure of dispersion. This, together with

a multiplication by 100, is actually what is done to obtain the coefficient of variation.

The summarized results are given in the table. It will be seen that it is divided into the three following sections, according to the method of treating the oil before the spectrophotometric examination: Section 1, containing the results from the untreated oil; 2, the results from the recommended saponification; and 3, results from an optional saponification treatment. At the bottom of the table is shown the coefficient of variation obtained with potassium chromate. This figure, 3.51, may be taken as the measure of performance from the instrumental standpoint and should therefore be continually kept in mind. In Section 1 of the table, in the third column, the average  $E_{1\text{cm}}^{1\%}$  values for the three oils is shown. In the fourth column are found the corresponding coefficients of variation. It will be observed that these coefficients are about twice those found in the potassium chromate standardization. It has been commonly believed that individual determination of extinction coefficient of a reference oil by each laboratory would lead to more nearly correct values for unknown oils. From the Table, Columns 1 and 2, it can be seen how this idea actually developed. Column 1 yielded an average value of 1008 units/gram and oil No. 3, 1740 units/gram when calculated from individual E values of the reference oil with corresponding coefficients of

*Results of 1939-40 U.S.P. Vitamin A collaborative spectrophotometric study on cod liver oils*

OIL NO.	AV. UNIT/G. ON 1ST U.S.P. CLO BASIS	CF-VAR. ON 1ST U.S.P. CLO BASIS	$E_{1\text{cm}}^{1\%}$ CM	CF-VAR. ON E VALUES	AVERAGE UNITS/G. IN NEW U.S.P. REF. OIL ON 1ST U.S.P. REF. CLO BASIS	AVERAGE CON- VERSION FACTOR 1ST U.S.P. CLO BASIS=3000 UNITS/GM
1. Raw Oil (Dissolved in 10-90 cyclopexane-absolute alcohol mixture)						
1	1008	5.79	0.519	7.08	1740	1941
2	3000	—	1.55	6.98		
3	1740	6.10	0.899	7.63		
2. A.O.A.C. and U.S.P. tentative procedure (saponification and extraction)						
1	1081	9.18	0.498	4.19	1707	2148
2	3000	—	1.40	5.47		
3	1707	9.53	0.795	9.23		
3. Any optional procedure (saponification and extraction)						
1	1055	6.78	0.471	11.6		
2	3000		1.34	7.87	1777	2245
3	1777	13.41	0.795	10.20		

\* Average Potassium Chromate Coefficient of Variation = 3.51.

variation of 5.79 and 6.10, which are slight improvements when compared with the coefficients of variation obtained from the extinction coefficients of the same oils (fourth column of table).

In Sections 2 and 3 of the table, however, it is evident that no improvement in calculating oil strength from individual determination of the extinction coefficient of the reference oil is possible. Upon first thought this may seem strange, but the answer may be that different oils behave differently in an equivalent saponification procedure. This supposition, together with the range of coefficients of variation from 4.19 to 13.41 found in Sections 2 and 3, indicates that the saponification procedure has definite possibilities but that at present it is not sufficiently exacting to warrant as much confidence in its use as in that of a dilution of the raw oil. With certain classes of products saponification procedures must be used but greater validity of results, as indicated by the coefficients of variation in Section 2, can then hardly be expected. Fortunately, it does not appear that even the largest coefficient of variation found in Section 2 would be sufficiently large to seriously interfere with the practical utility of such a saponification and extraction procedure.

Other figures in the table are of interest. Those in the last column are average conversion factors obtained under the titles indicating experimental conditions. It will be observed that the factor obtained with no saponification treatment is the smallest. With the recommended procedures the magnitude is intermediate, while with the optional saponification method the factor is largest. This situation indicates progressive losses of vitamin A with the above treatments.

The next to the last column in the table is of considerable immediate importance because these are the values found for the new U.S.P. reference oil. In this connection it is interesting to note that a tentative value of 1700 units/gram had previously been assigned to this oil, based primarily upon the biological findings.

#### SUMMARY

(1) In the spectrophotometric determination of vitamin A in cod liver oil better results are obtained by using a conversion factor based on direct comparison of the extinction coefficient of the unknown oil with the U.S.P. Reference Cod Liver Oil than by using a previously established conversion factor.

(2) While the specificity of the spectrophotometric determination of vitamin A may be increased by using a saponification process the variability of the results obtained is increased. While the results obtained in this particular study show no apparent advantage in using a saponification process recognition must be given to the fact that in some instances grossly erroneous results may be obtained if only the raw oil is examined.

(3) The magnitude of the error introduced by the saponification proc-

ess, while objectionable, does not appear to be sufficiently large to preclude its use for practical purposes.

(4) The maximum coefficients of variation found when optional saponification methods were used indicate the need for a definite saponification procedure.

(5) The magnitude of the coefficients of variation indicates that work on both the apparatus and the saponification procedure may be continued to good advantage.

(6) Even with the limitations indicated previously, there appears to be no technical reason for not tentatively accepting the spectrophotometric method for the assay of vitamin A.

It is recommended\* that studies on the determination of Vitamin A be continued.

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### REPORT ON VITAMIN B<sub>1</sub>

By O. L. KLINE (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

During the past year in the laboratory of the Associate Referee study was completed on a method of assay for vitamin B<sub>1</sub> suitable for products of relatively low vitamin B<sub>1</sub> potency. This method was presented before the Association in a paper entitled "An Improved Rat Growth Method for the Assay of Vitamin B<sub>1</sub> including Sulfite-Treatment of Dietary Constituents," by O. L. Kline, W. L. Hall, and J. F. Morgan. It is a rat growth procedure employing a diet demonstrated to be complete in all respects except for vitamin B<sub>1</sub>. Sodium bisulfite was used for the removal of vitamin B<sub>1</sub> from certain of the dietary constituents. Preliminary results indicate that a high degree of accuracy is possible with the method.

It is recommended\* that the rat growth method of assay for vitamin B<sub>1</sub> described by Kline, Hall, and Morgan be adopted tentatively, and be subjected to collaborative study during the coming year.

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No report on vitamin C was made by the associate referee.

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### REPORT ON VITAMIN D

THE USE OF REFERENCE COD LIVER OIL AND SKIM  
MILK AS THE REFERENCE SUBSTANCE.  
A UNIFORM SYSTEM OF SCORING

By WALTER C. RUSSELL (New Jersey Agricultural Experiment  
Station, New Brunswick, N. J.), *Associate Referee*

At the November, 1939 meeting of this Association, it was recommended that the method be revised so that the animals in the reference

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 49 (1941)

group receive in addition to the U.S.P. reference cod liver oil, non-vitamin D skim milk equal in quantity to the solids not fat in the volume of the milk fed the assay group, *This Journal*, 23, 341 (1940).

During the past year questionnaires were sent to 35 collaborators to ascertain the extent to which non-vitamin D skim milk was being used with the reference oil and whether the use of this reference substance, instead of the reference oil alone made any difference in the number of milks that were reported satisfactory or unsatisfactory. Of the 21 replies received, 3 showed no report, and by the remaining 18 collaborators some partial and some complete replies were submitted.

Eight collaborators reported the use of skim milk and the reference oil as the reference substance. One of these reported that the number of samples of milk passed was essentially the same whether reference oil alone or reference oil and skim milk were used as the reference substance. Another, who feeds a level of reference oil higher than that generally used, reported that fewer samples would have been declared satisfactory if skim milk and reference oil were used as the reference substance.

Four collaborators made a further comparison of the responses obtained with the reference oil alone and with the addition of skim milk. When the total quantity of reference oil used is of the order of 30 mg. the difference in response is small and it is likely that the number of samples of milk declared satisfactory or unsatisfactory would not be greatly affected. However, when the quantity of reference oil used is of the order of 50 mg., the difference in response is greater and the number of samples that would be declared unsatisfactory would be considerably larger than if a lower level of reference oil had been used. Therefore, when rats require a reference oil level of the order of 50 mg., an attempt should be made to produce animals that require a lower level in order that milk samples being assayed will not be penalized because a relatively large quantity of solids not fat must be fed with the reference oil.

Since there is considerable variation in the response of groups of rats fed the same test level of oil or milk, consideration should be given to a tolerance in comparing the response of a milk sample with that of the reference substance.

On several occasions the suggestion has been made that a uniform numerical system be agreed upon for expressing the degrees of healing obtained in vitamin D milk assays. Since the results for a milk are compared with a reference substance, it makes little difference how the results are expressed as far as the scientific worker is concerned. However, the various numerical systems that are used in making assay reports probably cause considerable confusion in the minds of public health officials, milk producers, and others who receive the reports.

Fifteen collaborators, who use the line test, reported the scoring systems they are using in a form suitable for comparative study. Ten systems

are in use by the fifteen collaborators. Ten of the fifteen collaborators use a range of scores from 0.0 to 4.0. In this range one uses 10 scores, four use 9, two use 8, one uses 7, and one uses 5. A narrow continuous line is given a value of 3.0 by two collaborators, a value of 2.0 by eight, and a value of 1.0 by three. Five use essentially the system, 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0, except that two of these do not use the 3.5 score. Hence a value of 2.0 for a narrow continuous line and 8 to 10 scores in a numerical range from 0.0 to 4.0 find the most extensive use.

If a uniform numerical system of scoring were adopted for the reporting of milk assay results it would be possible to state a tolerance for use in the interpretation of results.

Collaborators were practically unanimous in replying that they would be interested in considering the adoption of a uniform numerical system of expressing degrees of healing for the reporting of milk assays, and therefore it is recommended\* that further attention be given to this matter.

## REPORT ON VITAMIN K

### ASSAY BY CURATIVE BIOLOGICAL TEST

By H. J. ALMQUIST (Division of Poultry Husbandry, College  
of Agriculture, University of California, Berkeley, Calif.),  
*Associate Referee*

This assay is a comparison under specified conditions of the antihemorrhagic potency of any product with a chemically pure, standard, antihemorrhagic compound in controlling the blood prothrombin level of chicks. Since this is a first attempt at prescribing an official quantitative procedure, the reasons for the various steps in the method will be discussed in some detail.

Because of the fact that pure, synthetic substitutes or forms of vitamin K are now readily available, there is no longer any dependence on natural sources, and the necessity for standardization assays is correspondingly diminished.

### BASAL TEST RATION

The basal test ration is principally finely ground polished rice, chosen because this is the cleanest type of cereal available and is free of vitamin K or any extraneous material that may contain the vitamin. As a protein supplement to the polished rice, the most satisfactory product is sardine fish meal that has been extracted continuously with ethyl ether for 24 hours or more to remove small amounts of vitamin K. The fish meal also provides some of the mineral supplements. Casein is not so satisfactory as fish meal in this ration and does not allow as rapid growth. Rapid growth

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 49 (1941).



is desirable since it leads to earlier and more severe depletion of the chick in respect to vitamin K.

Dried brewer's yeast is used as a source of the water-soluble vitamins required by the chick. The yeast should be extracted with ethyl ether to remove possible traces of vitamin K. The full amount specified is needed to supplement the ration adequately in the well-known and less well-known vitamins and dietary factors of the water-soluble class required by chicks.

Cod liver oil U.S.P. is recommended as a source of vitamins A and D. Other types of animal feeding oils have been found to contain significant amounts of vitamin K and should not be used in vitamin K assay.

The basal ration developed in the Associate Referee's laboratory has been used with but minor modifications by the majority of laboratories engaged in vitamin K assay. The proportions of the ration are as follows:

	<i>per cent</i>
Sardine meal, ether extracted	17.5
Dried brewer's yeast, ether extracted	7.5
Ground polished rice	72.5
Cod-liver oil	1.0
Calcium carbonate	0.5
Salt, common (contains 0.5% Mn in form of the sulfate or carbonate).	1.0

This ration is probably deficient in vitamin E. However, the storage of this vitamin in chicks from hens fed practical diets is sufficient to carry the chicks over the short period required for a vitamin K assay. Addition of vitamin E to the ration is not advisable since some of the oxidation products of vitamin E that may be formed possess antihemorrhagic activity.

The ration is also deficient in the "gizzard factor," which is unrelated to vitamin K and does not affect growth rate. A satisfactory but expensive substitute for this factor is 0.5 per cent of cholic acid in the diet. This has not been found necessary in routine vitamin K assay.

#### CARE AND HOUSING OF TEST ANIMALS

Chicks are placed at hatching in metal, wire-mesh floored, electrically heated battery brooders. The temperature within the battery compartments should be automatically controlled and adjusted to 90–95° F. Food and water receptacles must be outside the compartment and available to the chick through apertures that permit only the head of the chick to pass. Fresh water should be provided daily in clean troughs. The diet should be renewed every 4 or 5 days or oftener. *If any portion of the diet becomes wet it must be discarded. Every care should be taken to prevent access of chicks to the droppings.* These precautions are necessary because of bacterial synthesis of the vitamin in wet feeds and in droppings. The chicks should be protected from excessive dust, especially that from dried green plants.

The chicks are maintained on the basal diet for 10–14 days, at which time there is almost invariably extreme depletion in vitamin K. A few (5%) of the chicks are then given the following rough test for depletion. A few drops of blood are withdrawn by making a small clean cut in an exposed wing vein at the junction of the ulna, radius, and humerus. The blood is caught in small vials and placed in a shaking device in a thermostat as described later for the determination of prothrombin clotting time. The time interval from withdrawal of blood to the formation of a firm clot is measured. At severe depletion the blood samples will not clot in less than 20 minutes and usually in not less than 30 minutes. There is little point in carrying such observations beyond 30 minutes since deteriorative processes in the exposed blood have undoubtedly begun and a long clotting time may not reflect the original condition of the blood.

The chicks used for this rough test are not given any supplement in the assay proper. Many of them will die from prolonged hemorrhage. Those that do not die may show a temporary decreased clotting time after considerable bleeding. Such chicks may be continued on the basal diet in a negative control group.

Chicks suffering from any form of severe hemorrhage should not be used in the test groups because extensive loss of blood tends to bring about a shortening of the blood-clotting time through some mechanism as yet unknown, but which appears to be independent of vitamin K.

After a satisfactory depletion period, the chicks are divided into groups of 10–12 and of reasonably uniform individual and group weight. Weak, sick, or precocious chicks should be discarded.

A group of 4 or 5 chicks of the same lot should be maintained on a practical chick mash containing at least 5 per cent of dried alfalfa and preferably not in the same battery as the chicks for assay groups. These chicks are to be used for standardization of the clotting agent, discussed later.

#### ADMINISTRATION OF SUPPLEMENTS

The majority of supplements that may be assayed can be given orally as a solution. A weighed quantity is dissolved in a counted number of drops from a medicine dropper so that the desired daily dose is contained in 1–4 drops. Each dropper is used only with one particular solution. Water solutions are preferable whenever possible.

When solution in a fat solvent is required, an inert carrier such as ethyl laurate should be chosen. Natural oils, such as cottonseed, peanut, and cod liver, should never be used. Such oils are susceptible to oxidation and are reactive in other ways. Their effects on the dissolved supplement can not be predicted. Some contradictory reports on different potencies of certain antihemorrhagic substances in water and in oils may be attributed to destruction of potency in the oil. The Associate Referee has observed total loss of activity of an antihemorrhagic preparation dissolved in cottonseed

oil. Mineral oils are not advisable because of their indigestibility, which offers a possibility that the animal may not extract all antihemorrhagic potency from the solvent. On the other hand, the potency of certain supplements has been found identical, whether they were administered in water or in ethyl laurate, and as long as the volume of the daily dose is small and the solvent is harmless there should be no difference in potency dependent upon the solvent.

Dosage of the chick is accomplished by holding the chick in one hand, head uppermost and mouth kept open by pressure of the thumb and forefinger at the corners. The required number of drops of test solution is allowed to fall well down the throat. After receiving supplements, chicks are not allowed access to food and water for one-half hour. Individual daily dosage is continued for 4 days at approximate 24 hour intervals.

The erratic results in the literature from short assay methods and the evidence that certain types of antihemorrhagic compounds develop maximum activity much more slowly than others is sufficient reason to reject hasty methods when attempting to prescribe a more quantitative assay procedure. For the present, the Associate Referee thinks that a definite level of intake should be maintained in the test animal for a sufficient period of time that potency modifying factors such as solubility, absorption, and metabolism have been able to attain a balance or plateau of influence on activity. It is quite possible that dosage for 4 days is longer than necessary. If the work of setting up an assay is carried out properly, however, an extra one or two days is only a small addition to the task and may lend greater precision.

#### MEASUREMENT OF SUPPLEMENT EFFECT

The effect of the supplements is determined on the fifth day at approximately 24 hours after the last dosage. At the same time, chicks that have received only the basal diet are tested as negative controls.

*Blood clotting time.*—The earliest quantitative measurement of supplement effect was the simple whole-blood clotting time, which is still used by some laboratories. If a sufficiently large number of chicks is included in each group, the average blood-clotting time for the group has a fairly close relation to the activity of the supplement.<sup>1,2</sup> Measurement of simple blood-clotting time can be conducted in a number of ways, one of which has been described previously.

There are, however, several serious objections to simple whole-blood clotting time, namely,

1. Blood-clotting time is known to be a resultant of the interaction of many components of the blood only one of which, prothrombin, is influenced by vitamin K. It is for this reason that the blood-clotting times of individuals receiving the

<sup>1</sup> Almquist, H. J., E. Mecchi, and A. A. Klose, *Biochem. J.*, **32**, 1897-1903 (1938).

<sup>2</sup> Almquist, H. J., and A. A. Klose, *Ibid.*, **33**, 1055-1060 (1939).

same vitamin K dosage are so highly variable. Blood-clotting time may be nearly normal when the blood prothrombin level is less than half the normal.

2. Blood-clotting time may be considerably influenced by the presence of tissue juice or extract. It is almost impossible to avoid contamination with tissue juice completely by any method of withdrawing blood samples that is not hopelessly laborious when applied to a large number of chicks.

3. Blood-clotting time may actually decrease in certain chicks after severe loss of blood. For this reason it is not advisable to test all chicks for blood-clotting time before administering supplements. In fact, some assay errors in the literature are a result of excessive bleeding rather than administration of a supplement that thus falsely appeared to diminish blood-clotting time.

4. Testing all depleted chicks also involves a high death rate. The Associate Referee has found no satisfactory way of preventing excessive bleeding from the necessary wounds.

5. Blood-clotting time is measured in periods from 2 to 30 minutes or longer. The longer clotting times tend to become more variable and to prolong the final collection of data. Furthermore, there is the question of deterioration in the blood samples when exposed overlong. It is often impossible to obtain any explicit values from certain chicks.

6. Blood-clotting time may vary considerably in consecutive samples from different parts of the same chick. This raises a doubt concerning the reliability of a blood sample consisting of only a few drops taken from one part of the chick.

*Whole-blood prothrombin time.*—For the above reasons, the Associate Referee and his associates abandoned blood-clotting time and adapted to vitamin K assay the "prothrombin-time" method of Quick, which measures more specifically the blood prothrombin level and, in turn, the effect of vitamin K supplements on the prothrombin level.<sup>2</sup> The method is described below:

For a clotting agent, an extract of chicken breast muscle is prepared. Approximately 10 grams of breast muscle from a chicken killed by bleeding is ground with sand and 10 ml. of 0.85% NaCl solution. The mixture is centrifuged and filtered on very coarse paper. The resulting liquid may be stored in a refrigerator for several days. It is preferable, however, to make a fresh tissue extract for each group of tests. Before use the extract is diluted with the salt solution to a concentration representing 5% of muscle, which concentration has proved ample. The diluted clotting agent (thrombokinase) at a concentration of 5% is then mixed with an equal volume of 0.025 *M* CaCl<sub>2</sub> solution. The mixture should clot the blood of normal chickens reared on the practical mash in 20–30 seconds when tested as directed in the present procedure. If it does not, the concentration of clotting agent should be altered until the prothrombin clotting times fall within this range. The blood of negative control chicks will usually fail to clot in less than 80 seconds.

Blood is rapidly and conveniently obtained by cutting off the head of the chick with scissors. The bird may be held in the left hand with the neck between thumb and forefinger in such a way as to direct the blood into a tube calibrated to 2 ml. and containing 0.2 ml. of 0.1 *M* Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> solution. When blood has been drawn to the 2 ml. mark, the tube is thoroughly shaken. Individual oxalated blood samples are obtained in this way from all chicks in a group. Slight contamination of the blood with tissue juice is of little importance in this method, since an excess of tissue juice (clotting agent) is added to the sample later.

Samples of 0.1 ml. of the oxalated blood are pipetted into small, flat-bottomed, cylindrical vials, 15×50 mm. A 0.2 ml. portion of the mixed thrombokinase-CaCl<sub>2</sub>

solution is run into the vial and a stop-watch started simultaneously. The vial is placed at once in a thermostat adjusted to 38.5°-39.0° C. A device is used to tilt the vial to an angle of approximately 45° and back to a vertical position once per second. The endpoint (clot formation) occurs very sharply. The watch is stopped when the bottom of the vial remains covered by a definite gelatinous film. The procedure is repeated to obtain a check measurement. Almost invariably the duplicate time measurements agree very closely, often to the second.

This improved method while requiring more preliminary preparation has several advantages over blood-clotting time, namely,

1. A larger sample of blood is used and is, therefore, more likely to be an accurate sample from the chick.
2. Measurements are made in seconds rather than minutes and the gathering of the final data is expedited. Determinations can be made in duplicate.
3. Conveniently measurable values can be obtained from every chick.
4. The variability of the results within a test group is much lower than that of the simple blood-clotting times, indicating that the effect of the supplement on the prothrombin level is more accurately and specifically measured.

TABLE 1.—*Means, standard errors, and coefficients of variability of simple blood-clotting times, and whole-blood-prothrombin times*

REF. STANDARD OF VITAMIN K PER KG. DIET	NO. OF CHICKS	BLOOD-CLOTTING TIME		WHOLE-BLOOD-PROTHROMBIN TIME	
		MEAN AND STAND- ARD ERROR	COEFFICIENT OF VARIABILITY	MEAN AND STANDARD ERROR	COEFFICIENT OF VARIABILITY
ml.		minutes		seconds	
3	15	10.07 ± 2.31	61.1	51.2 ± 1.9	17.1
6	15	3.42 ± 0.55	60.2	37.3 ± 1.0	9.6
12	15	1.97 ± 0.37	72.1	30.5 ± 0.5	6.1

Data on the comparative values and variations of blood-clotting time and prothrombin time are given in Table 1 to illustrate some of the statements made previously.

#### COMPUTATION OF RESULTS

It was found by the Associate Referee and associates that a plot of the reciprocal mean clotting time or the reciprocal mean prothrombin time against the logarithm of the vitamin K dosage yields practically a straight line.<sup>1,2</sup> In any assay it is desirable to establish this line by means of at least two groups fed different levels of a reference standard preparation. It is then easy to interpolate and calculate the potency of an assayed supplement in terms of the reference standard.

Omission of reference standard groups is a serious mistake. The response of chicks to a certain level of a standard source of activity is never quite the same in different lots of chicks and at different seasons of the year. For this reason, "master curves" and "response curves" for general use may be very misleading.

Certain assay methods proposed have been based on an erroneous as-

sumption that any vitamin K-deficient chick will give a standard response to a certain unit of activity. Furthermore, the units have been defined on a response basis rather than on a material basis. The Associate Referee and associates have deliberately refrained from defining a "unit" but have instead conducted all assays in comparison to a reference standard\* pending the establishment of a universal unit based on a pure compound. The history of certain other vitamins records the confusion attendant upon the definition of many "units." It was hoped that vitamin K would escape this same experience. However, workers later entering the field all hastened to present their particular definitions of "units." Fortunately these early "units" can now be considered obsolete.

#### PROPOSED TENTATIVE STANDARDS OF POTENCY

It has been suggested<sup>3</sup> that the highly potent compound, 2-methyl-1, 4-naphthoquinone, be adopted as a standard of activity and that 1 unit be defined as the antihemorrhagic activity of 1 microgram of this compound. Because of greater stability, 2-methyl-1, 4-naphthohydroquinone diacetate might serve as a better standard.<sup>4</sup> Both of these compounds are cheaply and easily prepared in a high state of purity, which can be tested conveniently by means of melting point. The melting point of the methyl naphthoquinone should be 105°–106° C., that of the diacetate 112°–113° C. The diacetate has the advantage of greater stability when mixed in the diet; this, however, is of little or no importance for oral administration. The comparative potencies of these and some other compounds and the exact method of calculating potencies from assay data can be explained best by example, as in Table 2 and Figure 1.

TABLE 2.—*Assay of certain antihemorrhagic compounds in comparison with methyl naphthoquinone*

SUPPLEMENT	DAILY DOSE	NUMBER OF CHICKS	AVERAGE PROTHROMBIN TIME	UNITS PER MILLIGRAM
	<i>micrograms</i>		<i>seconds</i>	
2-methyl-1,4-naphthoquinone	0.5	10	39.9	1000
2-methyl-1,4-naphthoquinone	2.0	10	22.9	1000
2-methyl-4-amino-1-naphthol hydrochloride ( $\frac{1}{2}$ mol. ethanol)	3.0	9	25.8	457
2-methyl-1,4-naphthohydroquinone diphosphoric acid ester (tetra sodium salt + 6 mol. water)	3.0	10	24.9	507

In Figure 1 these data are shown graphically and the manner of calcu-

\* A hexane extract of dried alfalfa equivalent in potency to 1 gram of dried alfalfa

<sup>3</sup> Thayer, S. A., S. B. Binkley, D. W. MacCorquodale, E. A. Doisy, A. D. Emmett, R. A. Brown, and O. D. Bird, *J. Am. Chem. Soc.*, 61, 2563 (1939).

<sup>4</sup> Ewing, D. T., J. M. Vandenbelt, and O. Kamm, *J. Biol. Chem.*, 131, 345 (1940).

lating the potencies of the tested compounds is indicated. Data on another assay are given in Table 3.

TABLE 3.—*Assay of certain antihemorrhagic compounds in comparison with methyl naphthoquinone*

SUPPLEMENT	DAILY DOSE	NUMBER OF CHICKS	AVERAGE PROTHROMBIN TIME	UNITS PER MILLIGRAM
	<i>micrograms</i>		<i>seconds</i>	
2-methyl-1,4-naphthoquinone	0.5	9	76.6	1000
2-methyl-1,4-naphthoquinone	2.0	7	31.0	1000
2-methyl-1,4-naphthohydroquinone diphosphoric acid ester (tetra sodium salt + 6 mol. water)	2.5	9	41.8	440
2-methyl-1,4-naphthohydroquinone diphosphoric acid ester (tetra sodium salt + 6 mol. water)	5.0	8	28.7	497

The activity of the 2-methyl 1, 4 naphthohydroquinone diphosphoric acid ester tetrasodium salt hexahydrate was found to be 440, 497, and 507 units per milligram.

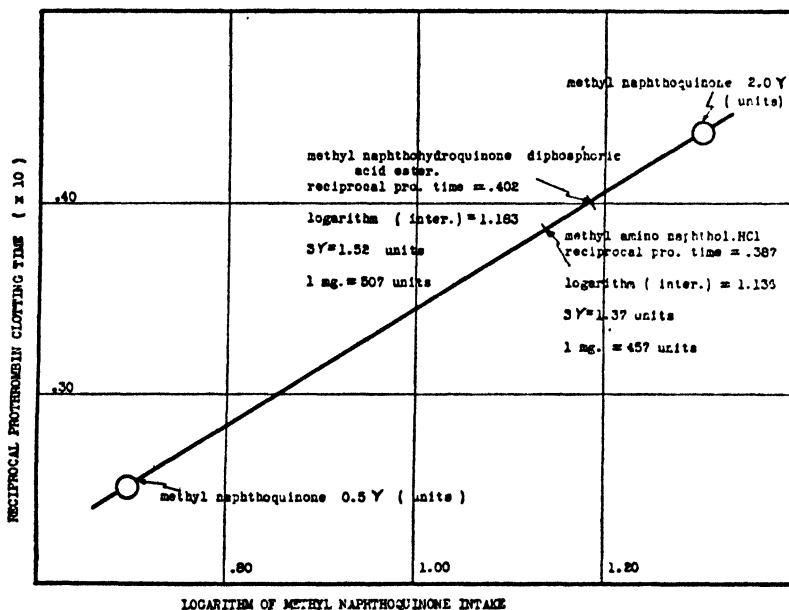


FIG. 1.—GRAPHICAL PRESENTATION OF DATA IN TABLE 2.

Repeated assays of 2-methyl 1, 4 naphthohydroquinone diacetate have yielded 470, 507, and 518 units per mg. It appears that the potency of this compound is close to one-half that of the methyl naphthoquinone.

The potency of vitamin K<sub>1</sub>, 2-methyl 3-phytyl 1, 4 naphthoquinone, from recent assays and as computed from earlier assays against the original reference standard (which has been accurately assayed in terms of methyl naphthoquinone) has been obtained as follows: 313, 308, 294, and 280 units per mg. As a general rule, repeated assays of the same substance will agree within a 10 per cent deviation range.

The method presented was adopted as tentative and published in *This Journal*, 24, 84 (1941). Subcommittee A also recommended that the method be studied collaboratively.

## REPORT ON RIBOFLAVIN

By A. R. KEMMERER (Agricultural Experiment Station,  
College Station, Texas), *Associate Referee*

Last year collaborative work was conducted for the first time on riboflavin, *This Journal*, 23, 346 (1940). The collaborators were asked to analyze a sample of dried skim milk and a sample of yeast by a colorimetric method submitted by the Associate Referee and by any other method with which they had had experience. The results obtained by the colorimetric method varied quite widely, and for the yeast sample they were much too high. A number of the collaborators determined the riboflavin by the bacteriological method of Snell and Strong<sup>1</sup> and by fluorometric methods. The Associate Referee recommended that further work be done on chemical methods, that fluorometric methods be considered, and that the Snell-Strong method be further studied.

The collaborators this year were asked to analyze two samples of feed: No. 1, yeast and No. 2, dried skim milk, by the three methods submitted and by any other method in which they might be interested. The methods submitted were an improved colorimetric method, the fluorometric method developed by Hodson and Norris,<sup>2</sup> and the microbiological technique of Snell and Strong.<sup>1</sup> These three methods are described in detail.

### RIBOFLAVIN

#### *Colorimetric Method*

#### REAGENTS

- (1) *Acid-70% methanol*.—Place 60 ml. of glacial acetic acid and 540 ml. of water in a 2000 ml. volumetric flask and dilute to volume with methanol.
- (2) *Sodium hydroxide*.—10%. Dissolve 10 grams of NaOH in 100 ml. of distilled water.
- (3) *Potassium permanganate*.—Use a saturated solution.
- (4) *Hydrogen peroxide*.—3%. Use U.S.P. grade.
- (5) *Glacial acetic acid*.
- (6) *Standard riboflavin*.—Weigh 20 mg. of Merck's pure synthetic riboflavin,

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 346 (1939).

<sup>2</sup> *J. Biol. Chem.*, 131, 621 (1939).



dissolve in 70% methanol containing 3% acetic acid (1) and dilute to exactly 1000 ml. Place a 20 ml. aliquot of this solution in a 100 ml. volumetric flask and dilute to volume (1). This solution is assumed to contain 4 p.p.m. of riboflavin.

(7) *Potassium chromate*.—0.02%. Weigh 0.2000 gram of  $K_2CrO_4$  and dilute to 1000 ml.

#### DETERMINATION

**Procedure A.—Yeast.**—Rigorously protect all operations from light. Weigh 10 grams of yeast and place in a 300 ml. Erlenmeyer flask. Add 75 ml. of the acid-methanol, mix thoroughly, and reflux gently in the dark for 1 hour. Cool, transfer to a 200 ml. graduated flask, make up to volume with methanol, and filter. Pipet a 50 ml. aliquot of filtrate into a 300 ml. Erlenmeyer flask and concentrate to about 10 ml. Add 10 ml. of water and filter into a 50 ml. graduated flask. Wash twice with 5 ml. portions of water. Add 2 ml. of glacial acetic acid and 5 ml. of the potassium permanganate solution to the filtrate, let stand 1 minute, and then shake vigorously 1 minute. Add 5 ml. of the  $H_2O_2$ , and shake vigorously. If the dark brown color caused by the potassium permanganate is not removed, add 1 ml. of glacial acetic acid and 3 or 4 ml. of  $H_2O_2$  and shake. Dilute to volume with methanol and filter. (The filtrate should be clear and have a yellowish green fluorescence.) If foaming occurs during any of the above procedure, break up the foam with a few drops of methanol.

**Procedure B.—Skim milk.**—Rigorously protect all operations from light. Weigh 10 grams of dried skim milk and reflux 1 hour with 75 ml. of the acid-methanol, as directed for yeast. Add 50 ml. of water, transfer to a 200 ml. volumetric flask, and dilute to about 165 ml. Add the NaOH solution dropwise until the casein comes down in a granular precipitate. Dilute to volume with water and filter. Place a 100 ml. aliquot of the filtrate in a 300 ml. flask and concentrate to about 25 ml. Add 1 ml. of glacial acetic acid and 2 ml. of the potassium permanganate solution, allow to stand 1 minute, and then shake 1 minute. Add 3 ml. of the  $H_2O_2$  and shake as directed for yeast. Dilute to 50 ml. with methanol and filter.

Compare the unknown solutions colorimetrically with a standard riboflavin solution containing 4 p.p.m. of riboflavin. Use the following equation to calculate the p.p.m. of riboflavin in the samples:

$$\text{p.p.m.} = \frac{4 \text{ mm. depth of standard}}{\text{mm. depth of unknown}} \times F.$$

F is the dilution factor. For the yeast F is 20 and for the skim milk it is 10.

Check on purity of riboflavin.

Compare the riboflavin solution containing 4 p.p.m. colorimetrically with the 0.02% potassium chromate and calculate the riboflavin equivalent of the chromate. Use the equation:

$$\text{p.p.m.} = \frac{4 \times \text{mm. depth of riboflavin solution}}{\text{mm. depth of potassium chromate}}.$$

#### Fluorometric method

##### REAGENTS

(a) *Sulfuric acid*.—Approximately 0.25 N. Dilute 5 ml. of  $H_2SO_4$  to 800 ml. with water.

(b) *Tri-sodium phosphate solution*.—Dissolve 65 grams of  $Na_3PO_4 \cdot 12 H_2O$  in 1 liter of distilled water

(c) *Acid acetone solution*.—3 volumes of commercial acetone+1 volume of

normal  $\text{H}_2\text{SO}_4$  (5 ml. of  $\text{H}_2\text{SO}_4$  in 200 ml. of water). This reagent is used only if products contain casein.

(d) *Sodium hydrosulfite solution*.—Dissolve 1 gram of ( $\text{Na}_2\text{S}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ ) and 1 gram of  $\text{NaHCO}_3$  in 20 ml. of ice-cold distilled water and keep in an ice bath. (This solution is stable for approximately 4 hours.)

(e) *Stannous chloride*.—Dissolve 10 grams of  $\text{SnCl}_2$  in 25 ml. of  $\text{HCl}$  and store in a brown, glass-stoppered bottle. For the determination dilute 1 ml. of the above stock solution with 200 ml. of water. Make a fresh preparation of the dilute solution daily.

(f) *Standard riboflavin solutions*.—Store in dark bottles in the refrigerator. Rigorously protect from light.

(1) *Solution A*.—Weigh 20 mg. of Merck's 100% pure synthetic riboflavin, dissolve, add a few drops of glacial acetic acid, and dilute to 500 ml. with water. (This solution is assumed to contain 40 p.p.m. of riboflavin.)

(2) *Solution B*.—Pipet 25 ml. of Solution A into a 100 ml. flask and dilute to volume. This solution is assumed to contain 10 p.p.m. of riboflavin.

(g) *Standard quinine sulfate solutions*.—Used to regulate the intensity of the activating light of a fluorometer. (They can not be used for visual comparisons of riboflavin. Sodium fluorescein, or a standard glass cube, may be substituted for quinine sulfate.)

*Solution A*.—Weigh 40 mg. of quinine sulfate, dissolve in about 20 ml. of 0.1  $N$   $\text{H}_2\text{SO}_4$  and dilute to 200 ml. in a graduated flask with the acid.

*Solution B*.—Pipet 10 ml. of Solution A into a 100 ml. graduated flask and make up to volume with 0.1  $N$   $\text{H}_2\text{SO}_4$ .

*Solution C*.—Pipet 10 ml. of Solution B into a 1000 ml. graduated flask and make up to volume with 0.1  $N$   $\text{H}_2\text{SO}_4$ . Solution C contains 0.2 p.p.m. of quinine sulfate.

#### CALIBRATION CURVE OF FLUOROMETER

(The directions given below are for calibration of the fluorometer manufactured by Pfaltz and Bauer Inc., New York. Other types of instruments may be used. Such instruments may not have the same sensitivity as the Pfaltz and Bauer instrument and therefore riboflavin solutions of different concentration than these may be needed.)

Pipet 20 ml. of the standard riboflavin Solution B(f)(2) into a 200 ml. graduated flask and dilute to volume (Soln. C). Dilute 40 ml. of Solution C to 50 ml. (Soln. D). Dilute 10 ml. of Solution B(f)(2) to 200 ml. (Soln. E). Dilute 40 ml. of Solution E to 50 ml. (Soln. F), 30 ml. of Solution E to 50 ml. (Soln. G), 20 ml. of Solution E to 50 ml. (Soln. H), 10 ml. of Solution E to 50 ml. (Soln. I), 5 ml. of Solution E to 50 ml. (Soln. J), and 0.0 ml. of Solution E to 50 ml. (Blank). Solution C contains 1.0 p.p.m. of riboflavin, Solution D 0.8, Solution E 0.5, Solution F 0.4, Solution G 0.3, Solution H 0.2, Solution I 0.1, and Solution J 0.05.

Place the quinine sulfate solution containing 0.2 p.p.m. (g) (3) in the cell of the fluorometer and adjust the iris diaphragm of the instrument to give a galvanometer deflection of 65 mm. Remove the quinine sulfate and obtain the galvanometer deflections for riboflavin solutions C, D, E, F, G, H, I, J, and blank. Also obtain the galvanometer deflections for the above riboflavin solutions for quinine sulfate deflections of 50.0, 33.0, and 18.2. Plot curve of concentration of riboflavin against galvanometer deflection for each deflection of quinine sulfate. Subtract the reading for blank from each value. Put all the curves on the same graph. By the above procedure a wide range of concentration of riboflavin can be read on the fluorometer.

#### CHECK ON PURITY OF RIBOFLAVIN

Obtain the galvanometer deflection for the quinine sulfate solution containing

0.2 p.p.m. (g) (3). Without changing the settings of the instrument obtain the galvanometer deflection for a riboflavin solution containing 0.2 p.p.m. (Soln. H above.) Report galvanometer deflections and filters used in the fluorometer.

#### DETERMINATION

##### *Procedure A—Yeast*

*For materials that do not contain casein.*—In all operations avoid exposure to light as much as possible. Weigh 5 grams of sample into a 300 ml. Erlenmeyer flask and pipet in 50 ml. of the 0.25 N  $\text{H}_2\text{SO}_4$ . Mix thoroughly and break up the lumps. Boil gently under a reflux condenser for 1 hour. Allow the sample to come to room temperature and remove from condenser. Bring the pH to 7.0–7.5 with the trisodium phosphate solution. Use phenol red or other suitable indicator. (30 ml. of the phosphate solution may be added before the solution is tested for pH.) Use only 1 drop of solution for each pH test. Transfer the solution to a 100 ml. graduated flask and make up to volume. Allow the mixture to stand 30 minutes and filter through a fluted filter. Pipet a 5 ml. aliquot into a 200 ml. volumetric flask and dilute to approximately 175 ml. with water. Add 2 ml. of the sodium hydrosulfite solution and 2 ml. of the stannous chloride solution. Make up to volume, mix well, and allow to stand for 10 minutes. Pour the solution into a 1 liter Erlenmeyer flask and shake vigorously for 5 minutes with access to air. Pipet 2 aliquots of 50 ml. each into 100 ml. Erlenmeyer flasks. Pour the remainder into a 100 ml. flask but do not measure it.

##### *Procedure B—Dried Skim Milk*

*For materials containing casein.*—Follow the same procedure except to use the acid-acetone solution for the extraction instead of  $\text{H}_2\text{SO}_4$ . Also use a 50 ml. aliquot for the reduction with the sodium hydrosulfite and stannous chloride.

Place a portion of the standard quinine sulfate solution in the cell of the fluorometer and adjust the iris diaphragm to give a galvanometer deflection of 65.0, 50.0, 33.0, or 18.2, according to the concentration of riboflavin expected in the unknown solution. Place part of the unknown solution in the fluorometer, obtain the galvanometer deflection, and calculate to riboflavin from the calibration curve. This is (A). To one of the 50 ml. portions of unknown add with a pipet 1 ml. of riboflavin solution (f) (2) and obtain the galvanometer deflection. Convert to riboflavin (B). To the remaining 50 ml. aliquot of the unknown add 1 ml. of the sodium hydrosulfite solution, mix well, and read the deflection. Convert to riboflavin (C). To 50 ml. of distilled water add 1 ml. of the riboflavin solution (f) (2) and obtain the deflection. Subtract the deflection given by distilled water from the reading and convert to riboflavin (D).

#### CALCULATION

Use the following formula to obtain the p.p.m. of riboflavin:

$$\left( A - 1.02C \right) \left( \frac{1.02D}{1.02B - A} \right) F = \text{p.p.m. of riboflavin.}$$

A, B, C, and D are as specified above and F is the dilution factor. For Sample I yeast F is 800 and for Sample II F is 80. No correction need be made for the deflection caused by the reagents in the unknown. It is eliminated in the above calculation.

##### *Microbiological Method*

(This method depends upon the use of a pure culture of *Lactobacillus Casei* €; contamination with other organisms invalidates results.)

## REAGENTS

(a) *Yeast extract*.—Dissolve 2 grams of Difco yeast extract in water and dilute to 100 ml.

(b) *Agar*.—Difco Bacto or some equally pure agar.

(c) *Yeast supplement*.—Dissolve 50 grams of Difco yeast extract in 250 ml. of water. Add 75 grams of basic Pb acetate (Horne's sugar reagent) dissolved in 250 ml of water. Centrifuge off the precipitate, add  $\text{NH}_4\text{OH}$  to the filtrate to a pH of approximately 10.0, and filter off the precipitate. Add glacial acetic acid to the filtrate until it is slightly acid, then pass in  $\text{H}_2\text{S}$  until the excess Pb is precipitated. Filter, and dilute filtrate to 500 ml. 1 ml. of this solution = 100 mg. of yeast extract. Add about 5 ml. of toluol to preserve the solution.

(d) *Peptone, photolyzed*.—Dissolve 20 grams of Difco Bacto peptone in 125 ml. of water. Dissolve 10 grams of  $\text{NaOH}$  in 125 ml. of water. Mix the two solutions, place mixture in a 9" crystallizing dish, and expose to light from a 100-watt bulb with a reflector at a distance of about 1 ft. for 6–10 hours. Then allow the mixture to stand 18–14 hours (24 hours in all). Neutralize the  $\text{NaOH}$  with glacial acetic acid. Add 3.5 grams of anhydrous sodium acetate and dilute to 400 ml. Add about 5 ml. of toluol to preserve this solution.

(e) *Potassium phosphate*.—Dissolve 25 grams of  $\text{K}_2\text{HPO}_4$  and 25 grams of  $\text{KH}_2\text{PO}_4$  in 250 ml. of water.

(f) *Mixed salts*.—Dissolve 10 grams of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 gram of  $\text{NaCl}$ , 0.5 gram of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.5 gram of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  in 250 ml. of water.

(g) *Cystine solution*.—Dissolve 0.5 gram in about 400 ml. of water containing about 3 ml. of  $\text{HCl}$ , and dilute to 500 ml. Add 5 ml. of toluol.

(h) *Glucose*.—Use anhydrous reagent grade.

(i) *Pure culture of Lactobacillus casei*  $\epsilon$ .—This culture may be obtained from Dr. J. V. Anzulovic, American Type Culture Collection, Georgetown University Medical School, 3900 Reservoir Rd., Washington, D. C. Ask for *Lactobacillus casei* No. 7469. The cost of one culture is \$2.00 plus 35¢ to cover cost of packing and postage.

(j) *Standard riboflavin solutions*.—Store in dark bottles in a refrigerator. Add 5 ml. of toluol for preservation. Protect from light.

*Solution A*.—Weigh 50 mg. of 100% Merck's synthetic riboflavin on an analytical balance and dilute to 1000 ml. with 0.02 *N* acetic acid (1.2 grams of glacial acetic acid to 1000 ml. of water). (This solution is assumed to contain 50 micrograms of riboflavin per ml.)

*Solution B*.—By means of a pipet place a 5 ml. aliquot of Solution A into a 250 ml. volumetric flask and dilute (in the dark) to the mark with 0.02 *N* acetic acid. (This solution is assumed to contain 1 microgram of riboflavin per ml.)

*Solution C*.—Pipet 10 ml. of Solution B into a 100 ml. volumetric flask and dilute to the mark with water. (This solution is assumed to contain 0.1 microgram per ml.) Discard solution after using.

(k) *Agar tube cultures (culture medium for Lactobacillus casei*  $\epsilon$ ).—Dissolve 0.5 gram of the glucose in 100 ml. of the yeast extract and add 1.5 grams of the agar. Heat the mixture in an autoclave or in a water bath until the agar is dissolved. Dilute to 100 ml. and mix well. Place 10 ml. portions in test tubes and plug with cotton. Sterilize in an autoclave at a pressure of 15 lbs. for 15 minutes. Allow the tubes to cool in an upright position.

(l) *Liquid culture medium*.—Mix 100 ml. of the photolyzed peptone, 100 ml. of the cystine solution, 10 grams of the glucose, 10 ml. of the yeast supplement, 5 ml. of the potassium phosphate, and 5 ml. of the mixed salts.

Test the pH and adjust to 6.6–6.8 if necessary by small additions of  $\text{KOH}$

(0.5%) or HCl (1+10). Use bromothymol blue as indicator. Dilute to 500 ml. If this medium is not used immediately, pipet 5 ml. portions into test tubes, plug with cotton, and autoclave at 15 lbs. pressure for 15 minutes.

(m) *Sodium chloride solution*.—Dissolve 9 grams of NaCl (pure) and make up to 1000 ml. Sterilize this solution when needed by placing 10 ml. in test tubes and autoclaving at 15 lbs. pressure for 15 minutes.

#### PREPARATION OF INOCULUM

(1) *Stock cultures*.—Make stab cultures into 3 or more of the agar tubes from the original pure culture of *Lactobacillus casei*  $\epsilon$ . Incubate at 37° C. for 24 hours and store in the refrigerator until they are needed for use. Keep one of the cultures (especially if future work is to be done) as a reserve stock culture and do not disturb it except to make new stock cultures from it at the end of 1 month. (The other stock tubes are to be used for the analyses and are good for 1 month only. If work is to be done after that time, make new stock cultures from the reserve stock culture.) Discard the cultures when 1 month old.

(2) *Sodium chloride inoculum*.—Pipet 5 ml. of the liquid culture medium into 6 test tubes (16×150 mm. to 20×150 mm.). To each culture medium tube add 1.0 ml. of riboflavin Solution B and 4 ml. of water (final vol. 10 ml.). Plug the tubes with cotton and sterilize by autoclaving at 15 lbs. pressure for 15 minutes. Avoid exposing the tubes to light at any time after riboflavin is added. After the tubes are cool, inoculate 3 with a stab from one of the stock cultures. Incubate 24 hours at 37° C. (Three cultures are made in order to have extra ones if any are lost.) Transfer 3 drops of the culture from one of the tubes into each of the remaining 3 tubes of liquid culture medium. Incubate 24–40 hours at 37° C. Centrifuge out the cells aseptically. Resuspend the cells from one of the tubes in 10 ml. of sterile 0.9% solution of NaCl. This culture is to be used as the inoculum and may be kept 5 days (store in refrigerator).

#### PREPARATION OF SAMPLE

(a) *Yeast or other materials containing over 20 p.p.m. of riboflavin*.—To 2 grams add 200 ml. of water, mix well, and autoclave at 120° C. for 15 min. Centrifuge off the insoluble matter and wash twice with 20 ml. of water, using the centrifuge. Combine the extracts and dilute to 1000 ml. in a graduated flask. 1 ml. of this extract = 2 mg. of the sample. Avoid exposure to light at all times.

(b) *Skim milk or other samples containing 10–20 p.p.m. of riboflavin*.—To 3 grams add 150 ml. of 0.1 N HCl. Mix well and autoclave 15 min. at 120° C. Adjust the pH to 6.6–6.8 with 0.1 N NaOH (bromothymol blue as indicator) and dilute to 500 ml. in a graduated flask. Assay this suspension directly. 1 ml. = 6 mg. of the sample. Avoid exposure to light.

#### PROCEDURE

With each set of assays run known quantities of riboflavin. Use duplicate tubes, each containing 0.0, 0.05, 0.075, 0.1, 0.15, 0.2, and single tubes, each containing 0.3 and 0.5 microgram of riboflavin. With a microburet, measure 0.0, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, and 5.0 ml. of the Standard Riboflavin Solution C into test tubes (specified above) and pipet in 5 ml. of the liquid culture medium. Add enough water to make the final volume 10 ml.

Use duplicate tubes for the two samples to be analyzed containing the equivalent of 0.5, 1, 2, 3, 4, and 5.0 mg. of yeast, or 1.5, 3, 6, 9, 12, and 15 mg. of dried skim milk. Measure 0.25, 0.5, 1.0, 1.5, 2, and 2.5 ml. of yeast extract and of the dried milk suspension into test tubes and pipet in 5 ml. of the liquid culture medium. Add enough distilled water to make the final volume 10 ml.

There are now 38 tubes in all, 14 containing the Standard Riboflavin Solution, 12 containing yeast extract, and 12 containing the dried milk suspension. Plug the tubes with cotton and sterilize in the autoclave at 15 lbs. pressure for 15 minutes. Allow to cool, and inoculate each with 1 drop of the 0.9% NaCl inoculum. Incubate at 37°C. for 3 days. Transfer contents to 125 ml. Erlenmeyer flasks, wash tubes with 10-20 ml. of distilled water, and titrate from a microburet to a pH of 7.0 with 0.1 N NaOH, using bromothymol blue as indicator.

#### CALCULATION OF RESULTS

Plot the micrograms of riboflavin against ml. of 0.1 N NaOH used in the titration of the standard riboflavin. If the blank is 1 ml. or over, the ingredients of the liquid culture contain riboflavin and the results should not be used. Then run the experiment again with reagents more rigorously freed from riboflavin.

From the volume of 0.1 N NaOH used in the titration for each level of yeast and of dried skim milk locate on the graph the corresponding micrograms of riboflavin. These values should be 0.05-0.25 microgram, otherwise they can not be trusted. If 3 of the values do not fall between this range, repeat the analysis with different quantities calculated from the results obtained. Calculate p.p.m. (micrograms per gram) for each quantity in the range specified (The results from each quantity should agree within 20% with the results from the other quantity.) Average the quantities in this range for the final result.

#### COLLABORATORS

The Associate Referee appreciates the generous cooperation of the following collaborators in this study.

- H. J. Prebluda, U. S. Industrial Chemicals, Inc., Baltimore Md.
- R. O. Brooke and S. Tyler, Wirthmore Research Lab., Malden, Mass.
- O. I. Struve, Eastern States Coop. Milling Corp., Buffalo, N. Y.
- S. Laufer, Schwarz Laboratories, Inc., New York City.
- J. S. Andrews, General Mills, Inc., Minneapolis, Minn.
- V. O. Wodicka and R. Clark, Purina Mills, St. Louis, Mo.
- H. R. Bird, University of Maryland, College Park, Md.
- A. Arnold, National Oil Products Co., Harrison, N. J.
- E. B. Vliet & E. Roedger, Abbott Laboratories, N. Chicago, Ill.
- F. Hafner, Archer-Daniels-Midland Company, Minneapolis, Minn.
- C. O. Gourley, The Beacon Milling Co., Inc., Cayuga, N. Y.
- B. L. Oser, Food Research Labs., Inc., Long Island City, N. Y.
- D. J. Hennessey, Fordham University, New York City.
- B. E. Lesley, California Packing Corp., San Francisco, Calif.
- W. J. Rudy, Allied Mills, Inc., Peoria, Ill.
- L. C. Norris, Cornell University, Ithaca, N. Y.
- O. L. Kline, Food and Drug Administration, Washington, D. C.
- W. L. Hall, Food and Drug Administration, Washington, D. C.
- J. C. Fritz, Borden & Co., Elgin, Ill.
- E. J. Lease & J. H. Mitchell, Clemson Agr. Coll., Clemson, S. C.

#### COMMENTS OF COLLABORATORS

*H. J. Prebluda.*—In order to avoid discrepancies in the bacteriological method, in future studies the Associate Referee should dispense cultures to all collaborators.

*R. O. Brooke and S. Tyler.*—The procedure given below for extracting riboflavin from samples is more simple than the one used in the fluorometric method sent out.

Weigh 1 gram of finely ground material into a 100 ml. fat flask; add 50 ml. of a solvent consisting of 25 ml. of normal  $\text{H}_2\text{SO}_4$  and 20 ml. of 95% ethyl alcohol diluted to 100 ml. with water; and reflux the mixture away from direct light at the temperature of boiling water for 1 hour. Allow the mixture to cool and filter through a dry Whatman No. 40, 11 cm. filter paper. To 5 ml. or 10 ml. aliquots of the clear filtrate add 70 ml. of distilled water. Adjust pH to 6.8 by means of Beckman pH meter and dilute to 100 ml.

By this procedure the dried skim milk ran 17 p.p.m. and the yeast 51 p.p.m. of riboflavin.

*O. I. Struve.*—In working with potassium chromate satisfactory color comparison on the Duboscq type colorimeter was not obtained with natural light. Artificial light from an ordinary incandescent lamp made the matching of colors easier.

*S. Laufer.*—On two samples of grassy feeds the results by the colorimetric method were more than twice as high as the results obtained by the bacteriological method. The bacteriological method is more reliable than the colorimetric.

*J. S. Andrews.*—In the colorimetric method the yeast extract was off-color.

*V. O. Wodicka and R. Clark.*—The colorimetric method is an improvement over the colorimetric method sent out last year. It is easier to handle and results in less off-colored solutions.

*H. R. Bird.*—Results by the colorimetric method are too high. The yeast samples yielded very satisfactory solutions for colorimetric readings, but the dried skim milk samples were slightly cloudy. The bacteriological method was very satisfactory.

*A. Arnold.*—More concentrated extracts of riboflavin are preferred in the bacteriological method than were recommended in the procedure studied.

*F. Hafner.*—Sodium fluorescein was used instead of quinine sulfate to standardize the setting of the fluorophotometer in the fluorometric method. The 0.2 p.p.m. of quinine sulfate solution did not give sufficient fluorescence to cause an appreciable deflection in the Pfaltz and Bauer fluorophotometer. The use of acetone along with  $\text{H}_2\text{SO}_4$  as an extract for the dried skim milk offered difficulty in filtration. The filtration was so slow that acetone was lost by evaporation.

*C. O. Gourley.*—Some work should be done on unextracted liver meal, fish meal, and alfalfa meal before any method for riboflavin is officially accepted.

*B. L. Oser.*—The bacteriological method provides a better measure of total riboflavin, free and combined, than do the other methods.

*B. E. Lesley.*—There is considerable difficulty in getting consistent readings with the Duboscq visual colorimeter for any colorimetric comparisons. A photoelectric colorimeter and spectrophotometer were used in the colorimetric method. With the photoelectric colorimeter the yeast ran 74.0 p.p.m. of riboflavin and the dried skim milk 19.7 p.p.m. With the spectrophotometer the yeast ran 63.4 p.p.m. and the dried skim milk 21.0 p.p.m. The samples also were run by the photometric method of Sullivan and Norris.<sup>1</sup> By this procedure the yeast contained 51.2 p.p.m. of riboflavin and the dried skim milk 16.2 p.p.m.

*L. C. Norris.*—The results obtained by the bacteriological and the fluorometric methods agree very well for the yeast but differ by about 15% for the dried skim milk. It is not unusual to obtain variations as wide as this, but the average variation between the two methods is probably not greater than  $\pm 5\%$ . In future studies a standard source of highly purified riboflavin free of moisture, to standardize the fluorometers used by the various workers and to be used as a reference in the bacteriological assays, is suggested.

*O. L. Kline.*—The work was carried out with the help of Mrs. E. P. Daniel. The bacteriological method was studied extensively in our laboratory during the past

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 535 (1939).

year. The results obtained on products in which riboflavin is present in solution are quite dependable. However, where it is necessary, with other types of materials, to resort to extraction procedures, the extraction method recommended by the Associate Referee has not always given dependable values. We have made considerable study of various methods of extraction. Results obtained on the yeast sample when various methods of extraction were used follow:

Method of extraction	Riboflavin p.p.m.
H <sub>2</sub> O—Autoclaved once	56.1
H <sub>2</sub> O—Autoclaved twice	65.4
Acid—Autoclaved once	58.7
Acid—Autoclaved twice	68.2

In the acid extractions, 2 grams of yeast was suspended in 200 ml. of 0.1 *N* HCl and autoclaved. Then the two samples were centrifuged, the solution decanted, and the residues washed with a small quantity of water. The combined extracts were neutralized with NaOH to pH of 6.8 and then diluted to 1 liter. When the sample was autoclaved twice, the material was centrifuged, the solution was decanted, and the residue was autoclaved with a second portion of acid before being washed and neutralized. The extraction with water was carried out in the same manner. From the data in the table, it seems that the acid extraction causes a significant though small increase in the amount of riboflavin extracted from the yeast. A second autoclaving appears to be a desirable modification of the extraction procedure for yeast.

The samples of dried skim milk and yeast were assayed by a rat growth procedure, the details of which will soon be published, *This Journal*, 24, 147 (1941). The riboflavin contents of the samples after a four-week period of assay are as follows:

	p.p.m.
Yeast	62
Dried skim milk	21

These values are in good agreement with those obtained in the bacteriological assay and may be of help in establishing the validity and general acceptance of the use of bacteria for assay purposes.

*W. L. Hall.*—A standard chromate solution used in last year's study was compared with a fresh chromate standard. The old standard, which was stored in a soft glass-stoppered bottle in a dark cabinet had a riboflavin equivalent of 7.84. The fresh chromate standard had a riboflavin equivalent of 7.92. This experiment indicates that a chromate standard should be useful and permanent.

*J. C. Früz.*—Difficulty was encountered with the microbiological method. The replicate determinations showed poor agreement, and some of the values obtained were obviously in error.

#### DISCUSSION OF RESULTS

From the results in Table 1 and from the comments of the collaborators it is readily seen that either the bacteriological method or the fluorometric method is more reliable than the colorimetric method. The results obtained by the colorimetric method for yeast are obviously too high. In the bacteriological method the mean riboflavin content of the dried skim milk is 19.9 p.p.m. The maximum variation from this mean is 24 per cent. Ten out of 16 of the collaborators obtained results within 10 per cent of



this mean, and 13 out of 16 obtained results within 15 per cent of the mean. The average riboflavin content of the yeast by the bacteriological method is 55.7 p.p.m., and the maximum variation from the mean is 36 per cent. Eight of the 16 collaborators obtained results within 10 per cent of the mean and 8 obtained results that were over 15 per cent from the mean.

TABLE 1.—*Results of collaborative study on riboflavin (p.p.m.)*

ANALYST	DRIED SKIM MILK				YEAST			
	BACTERIO- LOGICAL METHOD	FLUORO- METRIC METHOD	COLORI- METRIC METHOD	OTHER METHODS	BACTERIO- LOGICAL METHOD	FLUORO- METRIC METHOD	COLORI- METRIC METHOD	OTHER METHODS
1	16.8				54.0			
2	19.0			17.0	56.7			51.0
3	17.3		21.6		44.0		45.4	
4	19.7		24.1		53.9		69.6	
5	19.7		19.8		52.3		88.3	
6	21.6		21.1		65.9		76.8	
7	21.0		33.5		44.3		66.5	
8	20.1	19.7			54.2	50.9		
9	20.4				54.2			55.0
10		30.1				67.8		
11	20.5	20.2		21.4	49.0	48.1		
12	18.6	21.6			72.2	59.1		
13		21.4				50.3		
14			22.8	16.2			60.8	51.2
15	23.9				69.8			
16	15.1	17.8			37.6	37.2		
17	22.9		22.7	23.8	75.7		82.9	69.0
18			23.5				71.5	
19	21.0			21.0	54.5			62.0
20	21.0				53.0			
Average	19.9	21.8	23.6	19.9	55.7	52.2	70.2	57.6

Six collaborators determined the riboflavin content of the samples by the fluorometric method, and the mean was 21.8 p.p.m. for the dried skim milk and 52.2 p.p.m. for the yeast. For the skim milk the maximum variation from the mean is 38 per cent and for the yeast the maximum variation is 30 per cent. Four out of six of the collaborators obtained results that were within 10 per cent of the mean for the skim milk and 3 out of 6 did the same for the yeast.

One collaborator ran the samples by a rat growth method developed in his laboratory. The results obtained agreed very well with results obtained by the bacteriological method.

The results of this study are very promising, and it is quite evident that

either the bacteriological method or the fluorometric method can be used with a fair degree of accuracy for dried skim milk and yeast.

In Table 2 are given the riboflavin equivalents of 0.02 per cent potassium chromate. In this study the various collaborators were asked to compare the standard riboflavin solution they used in the colorimetric procedure with 0.02 per cent potassium chromate. The riboflavin equivalent varied from 4.32 to 7.71, which shows that the standards used were

TABLE 2.—*Riboflavin equivalent of 0.02%  $K_2CrO_4$*

ANALYST	RIBOFLAVIN EQUIVALENT
3	6.71
4	6.18
5	5.91
6	4.33
7	7.36
14	5.00
17	5.33
18	7.71
Mean	6.07

not all the same. Some of the riboflavin standard must have either contained impurities or the chromate and riboflavin colors were very difficult to match. The data in this table at least indicate that a primary standard should be developed for checking standard riboflavin solutions. The variation in standards used may account in part for the variation in results obtained by the various collaborators.

#### RECOMMENDATIONS\*

It is recommended—

(1) That both the bacteriological and fluorometric methods described in this report be tentatively adopted for the determination of riboflavin in yeast and dried skim milk.

(2) That studies on the bacteriological and fluorometric methods be extended to liver meal, fish meal, alfalfa meal, and other materials.

(3) That a primary standard for riboflavin be studied in order to have a method for checking the purity of riboflavin solutions used as standards.

#### REPORT ON CANNED FOODS

By V. B. BONNEY (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

L. M. Beacham conducted collaborative work on the determination of chlorides and total solids in tomato products. He also devised and rec-

\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 49 (1941).

ommended for collaborative study a method for the detection of added neutralizers in tomato products. No other collaborative work on methods of analysis for vegetables and vegetable products was done during the past year.

#### RECOMMENDATIONS\*

It is recommended—

(1) That Beacham's recommendation on methods for tomato products be adopted.

(2) That studies of methods for quality factors in fill of container studies be continued.

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#### REPORT ON TOMATO PRODUCTS

By L. M. BEACHAM (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Following the recommendations of the Referee on Canned Foods, made in the report for 1939, the Associate Referee again submitted authentic samples of tomato paste and tomato purée to collaborators for comparative results. They were requested to use the method for total solids given in *Methods of Analysis, A.O.A.C.*, 1935, 499, 16, and the rapid method for determining chlorides recommended for adoption as tentative at the last meeting. This method was published in *This Journal*, 23, 765 (1940). Samples of paste and purée were submitted to nine laboratories of the Food and Drug Administration and one commercial laboratory, with the request that a determination of total solids be made on each sample by three analysts, using different ovens or the same oven on different days. Determinations of the salt in duplicate by one analyst in each laboratory were also requested. The results (Table 1) indicate that duplicate determinations of total solids by the same analyst are in close agreement. Those made by different analysts on the same sample, while not agreeing so closely as duplicates by a single analyst, are satisfactory. A statistical study of these figures reveals that in only 5 cases out of 1,000 will an analyst obtain results on duplicate determinations that show a difference of more than 0.23 per cent in the case of paste and 0.16 per cent in the case of purée, while two workers, analyzing the same sample will obtain results within less than 0.31 per cent and 0.21 per cent for paste and purée, respectively, in 995 cases out of 1,000. The agreement between different laboratories when different cans were used was not so good as that among analysts within a laboratory working with the same can. This indicates that either the cans were not identical, or that the laboratories differed somewhat in the details of the procedure followed.

In order to clear up this point, another sample of purée and another sample of paste were obtained and sent to collaborators. The inspector

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 56 (1941).

who obtained these samples from the manufacturer was instructed to take 48 small cans of purée and an equal number of cans of paste. The cans were to be taken consecutively as they came from the closing machines

TABLE 1.—*Collaborative results on total solids and salt in tomato paste and purée*

LABORATORY	ANALYST	PASTE (%)				PURÉE (%)			
		SOLIDS		SALT		SOLIDS		SALT	
Cincinnati	A	25.71		.34	.34	15.59		.81	.81
	B	25.84				15.63			
	C	25.80				15.64			
New Orleans	A	25.72				15.39			
	B	25.58				15.14			
	C	25.63	25.68	.37	.36	15.53	15.43	.71	.82
St. Louis	A	25.38	25.43			15.24	15.27		
	B	25.51	25.53			15.27	15.34		
	C	25.63	25.67	.43	.42	15.23	15.27	.84	.85
Buffalo	A	25.56	25.56	.36	.37	15.40	15.42	.96	.96
	B	25.46	25.53			15.39	15.45		
	C	25.27	25.33			15.25	15.28		
New York	A	25.35	25.41			15.28	15.31		
	B	25.11	25.50			15.28	15.59		
	C	25.32	25.39	.37	.39	15.35	15.41	.83	.82
Food Division Washington, D.C.	A	25.18	25.32	.38	.36	15.36	15.40	.84	.84
	B	25.37	25.47			15.37	15.38		
	C	25.39	25.42			15.35	15.37		
Nat'l Cannery Association Washington	A	25.12	25.24			15.15	15.33		
	B	25.23	25.27			15.30	15.31		
	C	25.08	25.30	.40	.40	15.21	15.27	.82	.82
Philadelphia	A	25.05	25.11	.39	.40	15.08	15.12	.86	.86
	B	25.31	25.59			15.25	15.25		
	C	24.99	25.01			15.14	15.14		
Denver	A	25.27				15.02			
	B	25.01				15.18			
	C	25.11		.32	.35	15.31		.81	.84
San Francisco	A	25.22	25.27	.35	.36	15.08	15.15	.79	.80
	B	25.07	25.08			15.10	15.13		
	C	25.03	25.09			15.13	15.13		

and were to be numbered in the order taken. Great care was requested in order that the cans of each sample should be as uniform as possible. These samples were received and submitted to every Station of the Food and Drug Administration, and to Food Division. Each Station received 2 cans of paste and 2 cans of purée. The two cans of each product that each Sta-

tion received were always equidistant apart in the order in which they came from the closing machine; that is, the Station that received can No. 1 also received can No. 25, and the Station that received can No. 2 got

TABLE 2.—*Collaborative results on total solids in tomato purée*

CAN NO.	NAME OF STATION	ANALYST A		ANALYST B		ANALYST C	
1	St. Louis	10.14	10.13	10.14	10.11	10.14	10.14
2	Atlanta	10.21	10.24	10.30	10.25	10.24	10.18
4	Philadelphia	10.20	10.21	10.18	10.19	10.22	10.22
5	New York	10.25	10.22	10.22	10.22	10.17	10.17
6	New Orleans	9.91	9.97	10.13	10.21	10.28	10.26
7	Los Angeles	9.96	10.00	10.06	10.06	10.01	10.04
8	Denver	10.09	10.08	10.10	10.06	10.14	10.08
9	San Francisco	9.80	9.90	9.91	9.94	10.03	9.97
11	Boston	10.05	9.99	9.94	9.97	9.80	9.76
12	Buffalo	10.04	10.07	10.07	10.10	10.06	10.09
13	Chicago	9.77	9.90	10.07	9.95	9.86	9.85
14	Cincinnati	10.05	10.02	10.02	10.00	10.06	10.03
15	Kansas City	10.14	10.11	10.20	10.10	10.10	10.04
16	Minneapolis	10.19	10.17	10.13	10.12	10.21	10.21
17	Food Division	10.30	10.27	10.31	10.33		
18	Food Division	10.32	10.28	10.34	10.32		
19	Food Division	10.32	10.30	10.35	10.34		
20	Food Division	10.30	10.22	10.20	10.25	10.28	10.27
21	Food Division	10.35	10.32	10.37	10.29		
22	Seattle	10.23	10.25	10.21	10.23		
23	Food Division	10.30	10.29	10.31	10.20		
24	Baltimore	10.31	10.31	10.29	10.33		
25	St. Louis	10.23	10.22	10.20	10.21	10.17	10.22
26	Atlanta	10.23	10.23	10.25	10.22	10.25	10.19
28	Philadelphia	10.23	10.24	10.22	10.22	10.21	10.21
29	New York	10.20	10.18	10.18	10.11	10.19	10.18
30	New Orleans	10.35	10.40	10.57	10.58	10.52	10.56
31	Los Angeles	10.15	10.16	10.38	10.34	10.24	10.23
32	Denver	10.26	10.25	10.39	10.31	10.26	10.27
33	San Francisco	10.18	10.18	10.28	10.30	10.36	10.35
35	Boston	10.27	10.23	10.22	10.17	10.05	10.10
36	Buffalo	10.25	10.28	10.30	10.27	10.28	10.27
37	Chicago	10.19	10.19	10.34	10.19	10.29	10.19
38	Cincinnati	10.25	10.25	10.19	10.20	10.28	10.28
39	Kansas City	10.31	10.25	10.39	10.32	10.35	10.32
40	Minneapolis	10.23	10.23	10.24	10.21	10.31	10.28
41	Food Division	10.21	10.21	10.21	10.20		
42	Food Division	10.24	10.22	10.28	10.29		
43	Food Division	10.19	10.18	10.20	10.15		
44	Food Division	10.31	10.31	10.30	10.27	10.30	10.28
45	Food Division	10.18	10.19	10.21	10.19		
46	Seattle	10.24	10.25	10.30	10.20		
47	Food Division	10.21	10.21	10.24	10.22		
48	Baltimore	10.35	10.32	10.37	10.35		

can No. 26, etc. Each Station was requested to have three analysts make duplicate determinations on each can. No two analysts were to use the same oven at the same time. The method to be used is the procedure given in *Methods of Analysis, A.O.A.C.*, 1940, 520, 18.

The results obtained by the collaborators are given in Table 2 for purée and in Table 3 for paste. These results indicate that despite the precaution taken to insure uniformity between the cans in the sample, differences did exist so that those cans in the beginning of the series had lower solids contents than those towards the end of the series. Thus it is not possible to show complete agreement among all the Stations on all of the cans analyzed. With analysts working on the same can, however, there is close agreement. Some analysts making the analyses in aluminum dishes that had been allowed to stand several days in the ice box reported presence of mold or, in one case, corrosion. A study of this condition indicated that the white spots that had been considered to be mold were floating masses of aluminum salts. The results of all analysts reporting this "moldy" condition have been eliminated from the tables. No opportunity was had to make statistical studies of the results given in Tables 2 and 3, but they appear to follow the pattern exhibited by the results for solids given in Table 1, and are sufficiently close to support the adoption of the method as official. This method is given in *Methods of Analysis, A.O.A.C.* as being applicable to canned products only, but there appears to be no reason why it should be so restricted.

#### COLLABORATORS

Thanks are extended to the following collaborators who assisted in the determinations reported in Tables 1, 2, and 3: J. P. Alden, S. Alfend, H. W. Ayres, H. P. Bennett, S. M. Berman, H. M. Boggs, H. Bois, H. M. Bollinger, A. G. Buell, J. Carol, L. H. Chernoff, H. Cohen, H. W. Conroy, T. C. Dunn, R. T. Elliott, S. B. Falek, L. H. Feldstein, L. W. Ferris, S. D. Fine, N. M. Foote, N. E. Foster, F. M. Garfield, H. W. Gerritz, M. J. Gnagy, C. A. Greenleaf, H. D. Grigsby, A. W. Hanson, W. K. Hays, W. Horwitz, A. Hunt, R. Hyatt, M. M. Jackson, C. R. Joiner, L. Jones, O. S. Keeney, T. O. Kellems, W. H. King, G. Kirsten, G. Q. Lipscomb, J. H. C. Loughrey, H. I. Macomber, M. Matluck, D. W. McLaren, K. F. McClure, H. J. Meuron, P. A. Mills, W. R. Moses, S. C. Oglesby, R. E. O'Neill, J. I. Palmore, N. H. Sandborn, J. A. Schuldiner, I. Schurman, G. Smith, H. R. Smith, J. B. Snider, C. B. Stone, T. E. Strange, J. A. Thomas, M. Tubis, H. G. Underwood, M. D. Voth, W. W. Wallace, J. F. Weeks, Jr., L. C. Weiss, D. H. Williams, W. C. Woodfin, D. A. York.

Pertinent comments on the method are as follows:

*E. C. Boudreaux, New Orleans.*—There was in the minds of our chemists a question as to just what was meant by "apparent dryness." Was it lack of fluidity, or should the material appear to be devoid of any droplets of moisture? In the case

of these samples, however, we judge "apparent dryness" meant the elimination of all visual evidence of moisture.

C. W. Harrison, *Minneapolis*.—The matter of what constitutes "apparent dryness" leaves too much to the matter of individual judgment.

TABLE 3.—*Collaborative results on total solids in tomato paste*

CAN NO.	NAME OF STATION	ANALYST A		ANALYST B		ANALYST C	
1	Minneapolis	26.41	26.56	26.35	26.30	26.52	26.67
2	Kansas City	26.41	26.39	26.38	26.31	26.34	26.31
3	Cincinnati	26.61	26.57	26.43	26.44	26.57	26.64
4	Chicago	26.28	26.34	26.27	26.35	26.31	26.32
5	Buffalo	26.43	26.45	26.50	26.54	26.54	26.39
6	Boston	26.24	26.31	26.08	26.26	26.09	26.12
8	San Francisco	26.28	26.29	26.64	26.56	26.89	26.72
9	Denver	26.71	26.51	26.56	26.62	27.14	27.39
10	Los Angeles	26.33	26.38	26.36	26.40	26.61	26.61
11	New Orleans	26.61	26.75	27.30	27.32	27.20	26.86
12	New York	26.17	26.50	26.45	26.20	26.38	26.27
13	Philadelphia	26.58	26.64	26.51	26.42	26.37	26.34
15	Atlanta	26.26	26.19	26.34	26.31	26.39	26.35
16	St. Louis	26.22	26.19	26.12	26.21	26.24	26.30
18	Seattle	26.13	26.19	26.28	26.28		
20	Baltimore	26.63	26.55	26.56	26.60	26.40	26.42
24	Food Division	27.05	27.17	27.10	27.19	27.40	27.26
25	Minneapolis	27.09	26.96	26.97	26.98	27.24	27.15
26	Kansas City	27.09	27.06	27.14	27.06	26.98	26.87
27	Cincinnati	27.02	26.98	27.04	27.06	27.06	27.05
28	Chicago	27.11	27.12	26.79	26.88	26.97	26.97
29	Buffalo	27.02	26.98	26.89	27.11	27.02	27.06
30	Boston	27.04	26.92	26.78	26.65	26.72	26.71
32	San Francisco	26.89	26.89	27.17	27.14	27.38	27.43
33	Denver	27.11	27.08	27.26	27.31	27.67	27.97
34	Los Angeles	26.80	26.83	26.94	26.86	26.76	Lost
35	New Orleans	27.11	27.16	27.69	27.80	27.37	27.30
36	New York	26.75	26.85	26.95	26.81	26.81	26.79
37	Philadelphia	26.99	26.97	26.91	27.04	27.11	27.06
39	Atlanta	26.99	26.91	26.85	26.86	26.83	27.06
40	St. Louis	26.81	26.80	26.77	26.71	26.92	26.85
41	Food Division			27.07	27.19		
42	Seattle	26.81	26.86	26.80	26.90		
43	Food Division			26.95	26.97		
44	Baltimore	27.31	27.21	27.32	27.24	27.12	27.13
45	Food Division			27.04	27.06		
46	Food Division			27.04	27.08		
47	Food Division			27.05	27.04		
48	Food Division	27.06	26.95	26.96	27.00	27.07	26.94

M. J. Gnagy, *Los Angeles*.—(1) It is believed that bubbling the air through the  $H_2SO_4$  during the preliminary drying, especially during the first hours, is not necessary. A faster flow of air and a faster removal of moisture undoubtedly could be maintained without the danger of carrying over acid into the oven. (2) It is also

believed that if a definite quantity of water is added to dilute the purée (or the paste) when spreading the material uniformly over the bottom of the dish, the time of the preliminary drying will be shortened, the drying of the material will be more uniform, and consequently a better check in results will be secured.

*A. M. Henry, Atlanta.*—In order to determine how much of the purée and paste to weigh out, it was thought desirable to make a refractometer reading in order to determine the amount of solids. This can be determined roughly by adding 10% to the soluble solids or by referring to Sipple's table in Food Research, Volume 1, No. 2.

From the results obtained and observations of the procedure at the Atlanta Station, it would appear that one cause of inaccurate results may be due to loss in weight of the portion being weighed out due to slowness of the operator.

The results of the analyses for salt, obtained with the rapid method, are, with one exception, in close agreement and, as may be seen in Table 1, show that the method may be depended upon.

A study was undertaken during the year to develop a method to determine the presence of neutralizing agents in tomato paste. The following method was devised:

Weigh 25 grams of paste, transfer to 250 ml. volumetric flask with alcohol, and make to volume with 95%  $C_2H_5OH$ . Use care to keep the alcohol concentration high, otherwise all of the pectin will not precipitate. Shake well and filter without washing through a cotton filter. Warm a small portion of the filtrate gently with activated charcoal (Nuchar was used in this experimental work) in an Erlenmeyer flask, guarding against loss by evaporation; cool, and filter the clarified liquor. Pipet 25 ml. into a 300 ml. Erlenmeyer flask, add 100 ml. of water, and titrate with 0.1 *N* NaOH, using phenolphthalein as an indicator. (This determination indicates the free acid present in the tomato paste.)

Pipet 25 ml. of the alcoholic filtrate into a centrifuge bottle. Add a quantity of 0.1 *N*  $Pb(C_2H_3O_2)_2$  slightly greater than the quantity of 0.1 *N* NaOH required to neutralize the free acid. Centrifuge gently and add to the clear supernatant liquor a drop of the lead acetate solution. If a precipitate forms, add 1 ml. of lead acetate solution, centrifuge, and repeat until no further precipitate forms upon the addition of 1 drop of lead acetate solution. When sufficient lead acetate has been added, centrifuge for 10 minutes at 1800 r.p.m., decant the clear liquor, and drain. Add 50-100 ml. of 80%  $C_2H_5OH$ , shake well, and again centrifuge for 10 minutes at 1800 r.p.m. Decant the liquor and drain. Add 100 ml. of water, shake well, and pass in  $H_2S$  to saturation. Filter, and wash with hot water. Boil the filtrate to expel  $H_2S$ , cool, and titrate with 0.1 *N* NaOH. This titration determines the total organic acid that was present in the paste, either as free acid or as organic salts, as well as any HCl that may have been generated from the NaCl present. After titrating, add 2 ml. of  $HNO_3$  and a measured quantity of 0.1 *N*  $AgNO_3$  in excess of that required to precipitate all the chlorides present. Boil gently to coagulate the  $AgCl$  precipitated, cool, and back-titrate with 0.1 *N*  $NH_4CNS$ , using ferric alum as an indicator. Subtract the amount of 0.1 *N*  $AgNO_3$  used in precipitating the chlorides from the 0.1 *N* NaOH titer. The remainder is the amount of 0.1 *N* NaOH required to neutralize the total organic acid present in the paste, both free and combined. The use of Nuchar does not appear to affect the determination of free acid in the alcoholic filtrate, since identical results were obtained before and after clarification. The end point in titrating the unclarified solution is somewhat obscure, however, which makes the use of a clarifying agent desirable.



Results obtained by the use of this method show that in authentic tomato paste small amounts of combined acid are present. Much additional work will be necessary before results can be so interpreted as to indicate whether or not a portion of the free acid has been neutralized in the manufacture of the paste.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the parenthetical expression "(Applicable to canned products only)" be eliminated from the tentative method for the determination of total solids in tomato products given in *Methods of Analysis, A.O.A.C.*, 1940, 520, 18.

(2) That this method, so revised, be made official, first action.

(3) That the present official method for the determination of total chlorides in tomato juice be dropped, final action.

(4) That the method for determining free and combined acids in tomato products described in this report be submitted to collaborative study with a view to developing it into a means of detecting the use of neutralizing agents in tomato paste.

#### NEW ASSISTANTS TO REFEREES

The following changes and additions have been made since the list was published in *This Journal*, 24, 6 (1941).

W. L. Taylor of the Archer-Daniels-Midland Co., Chicago, Ill., was appointed assistant to the Referee on Cereals to study soya products, in place of J. W. Hayward.

Robert L. Herd of the U. S. Food and Drug Administration, Washington, D. C., was appointed assistant to the Referee on Miscellaneous Drugs to study the subject of quinine and strychnine.

W. C. Woodfin of the U. S. Food and Drug Administration, Atlanta, Ga., was appointed to assist the Referee on Miscellaneous Drugs and to study alkaline metals.

Eugene H. Wells of the U. S. Food and Drug Administration was appointed as assistant to the Referee on Synthetic Drugs for the study of acetanilid.

R. G. Snow of Igleheart Brothers, Inc., Evansville, Ind., was appointed to assist the Referee on Cereals in the study of H-ion concentration.

Otto A. Bessey of the Harvard Medical School, Boston, Mass., was appointed as assistant to the Referee on Vitamins to study vitamin C.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 56 (1941).

## ERRATA AND EMENDATIONS

*Methods of Analysis, A.O.A.C., 1940*

Page	Section	
46	9, line 1	Change "31 g" to "13 g."
504	46, 2nd equation	Change "8460" to "8640" and "9.51" to "95.1."
517	Ref. 26	Change "28" to "38."
608	146(c)	After word "of," add "KBrO <sub>3</sub> and 12 g of."
667	6, last col.	Change "31.4" to "41.4."
729	Basic phosphate slag, 6th line	Change "62 and 64 or 65" to "67 or 68".
740	Bromide in mineral water	Change "521" to "541."

The report on Changes in Methods, *This Journal*, 24, 66 (1941), should also be consulted.

*This Journal*, 24 (1941)

Pages 66 and 75 (16), line 2 and (2), line 2, respectively, delete reference to section 11.

Page 68, in heading for zinc method change "maximum" to "minimum."

Page 81, (7), change "20 ml." to "30 ml."

Page 82, last line under "Determination," insert word "not" after the word "is."

Page 87, Soln A.—In line 2 change "12 g." to "1.2 g."

## CONTRIBUTED PAPERS

### OBSERVATIONS ON THE CHICK METHOD FOR THE ASSAY OF VITAMIN D

#### II. A MODIFIED BASAL RATION\*

By HENRY W. LOY, JR.,† JAMES B. DEWITT, and LILA F. KNUDSEN‡ (U. S. Food and Drug Administration, Washington, D. C.)

An earlier report<sup>1</sup> from this laboratory described studies of the tentative A.O.A.C. chick method for the assay of vitamin D<sup>2</sup>, and dealt with the ashing procedure and the relationship of body weight to per cent bone ash of the chicks. Further critical studies of the method have been directed toward the development of a modified basal ration designed to decrease the variability of the results obtained in the present procedure. Since it appeared to be possible that variations in the degree of calcification shown in successive experiments might be due, in part, to variations in dietary constituents, the use of whole grain products rather than milled by-products was suggested to eliminate changes in composition. It has been found in the studies here reported that by the use of such ingredients of uniform composition and ready availability, and by the readjustment of mineral content it is possible to obtain increased uniformity of response, as reflected by smaller degree of variation from the average per cent bone ash and by greater spread in per cent bone ash between levels of vitamin D intake.

#### EXPERIMENTAL

A series of preliminary investigations involving a number of rations was conducted. Rations were devised that varied in type of constituents, in percentage composition, and in calcium and phosphorus levels and ratios. Such dietary materials as meat products, skim milk powder, alfalfa leaf meal, and various cereal grains were included.

The bone ash response of the chicks on each of the rations was determined in experiments in which chicks were fed each ration for a three-week period with and without vitamin D. As a result of these investigations, the ration shown to be the most promising, designated as No. 13 (Table 1), was selected for further study. Comparisons were then made of the responses in bone ash of chicks fed the A.O.A.C. ration and ration No.

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† Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Georgetown University, Washington, D. C.

‡ Acknowledgment.—The authors are indebted to Dr. M. X. Sullivan, Georgetown University, and to Drs. E. M. Nelson, C. D. Tolle, and O. L. Kline, U. S. Food and Drug Administration, for their suggestions and assistance.

<sup>1</sup> *This Journal*, 24, 190 (1941).

<sup>2</sup> *Ibid.*, 22, 81 (1939); *Methods of Analysis*, A.O.A.C., 1940, 371.

13 in nine separate experiments in which the procedure prescribed by the A.O.A.C. method was used.

TABLE 1.—*Comparison of basal rations*

A.O.A.C.		BASAL RATION		NO. 13	
<i>Ingredient</i>	<i>per cent</i>	<i>Ingredient</i>	<i>per cent</i>		
Ground yellow corn	58	Ground yellow corn	54		
Wheat flour middlings	25	Ground whole wheat	20		
		Ground rolled oats	10		
Crude domestic acid-ppt'd casein	12	Crude domestic acid-ppt'd casein	11.5		
Nonirradiated yeast (7% min. N)	2	Nonirradiated yeast (7% min. N)	2		
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> (ppt'd)	2	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> (ppt'd)	0.5		
		CaCO <sub>3</sub> (ppt'd)	1		
NaCl (0.02% KI)	1	NaCl (0.02% KI)	1		
	100		100		
Add 0.2 gram of MnSO <sub>4</sub> ·4H <sub>2</sub> O		Add 0.2 gram of MnSO <sub>4</sub> ·4H <sub>2</sub> O			
per kg. of mixture		per kg. of mixture			
APPROXIMATE ANALYSIS		APPROXIMATE ANALYSIS			
	<i>per cent</i>		<i>per cent</i>		
Protein	21.6	Protein	20.7		
Fat	3.8	Fat	3.3		
Fiber	2.4	Fiber	2.1		
Ash	5.1	Ash	4.3		
N-free extract	56.3	N-free extract	58.9		
Ca	0.87	Ca	0.69		
P	0.87	P	0.51		
Ca:P = 1:1		Ca:P = 1:0.74			

Day-old chicks were divided into two lots by random selection, and each lot was fed one of the rations under study. The lots were subdivided into groups of approximately 20 chicks, and these groups were then fed different levels of vitamin D intake. In 4 of the 9 experiments, levels of 0 and 15 units of vitamin D per 100 grams of feed were used. In one experiment the levels fed were 0, 5, 10, and 15 units; and in the remaining 4 experiments the 0, 5, 10, 15, and 20 unit levels were fed. At the end of the three-week assay period the chicks were weighed and killed and ash determinations were made on the moisture-free, fat-free tibiae. The results for each ration were then compared in regard to response in per cent bone ash to different levels of vitamin D intake, uniformity of response of individual chicks, and relationship of per cent bone ash to body weight. In addition, observations were made on the severity of gizzard erosion of the type described by Bird *et al.*<sup>3</sup>

During the preliminary investigations it was observed that approximately 90 per cent of the day-old chicks were affected with slight gizzard

<sup>3</sup> *J. Nutrition*, 12, 571 (1936).

erosion, and that many chicks on the A.O.A.C. ration showed severe gizzard erosion at the end of the three-week assay period. It appeared that such a high degree of gizzard erosion might affect individual variations in bone ash percentages by causing other abnormalities not readily discernible in routine examination. Therefore, in 2 of the experiments that involved the 0, 5, 10, 15, and 20 unit levels of vitamin D, the average index of gizzard erosion was determined for all chicks regardless of weight. The gizzards were removed, washed, and examined immediately after the chicks were killed. The index of erosion was based upon the extent and the severity of the lesions.

The average total spread in per cent bone ash between the 0 and 15 unit levels of vitamin D intake was determined in all the experiments. For this purpose, group ash determinations were made on the left tibiae of chicks weighing more than 100 grams.

The significance of the mean difference between the per cent bone ash of chicks weighing more than 100 grams and of all chicks regardless of weight was determined in 7 of the experiments, 4 of which included the 0, 5, 10, 15, and 20 unit levels of vitamin D intake, and the other three the 0 and 15 unit levels. The right tibiae of all chicks regardless of weight\* were ashed individually. In these same experiments, the data obtained by ashing individually the right tibiae of all chicks regardless of weight were used to determine the significance of the mean difference between the standard deviations of the per cent bone ash for the two rations.

In order to compare the results of the group (calculated) and individual ashing procedures, data were used from the 4 experiments that included the 0, 5, 10, 15, and 20 unit levels of vitamin D intake. Individual ash determinations were made on the right tibiae of all chicks regardless of weight, while group ash was calculated from the total bone and total ash weights of these same tibiae.<sup>4</sup> Data from this same group of experiments were used to determine the significance of the difference in per cent bone ash of the chicks between adjacent levels of vitamin D. Also, the relationship of per cent bone ash to body weight was determined for all chicks included in each of the 5 respective levels of vitamin D fed.

## DISCUSSION OF RESULTS

As shown in Table 2, the average index of gizzard erosion per chick increased throughout the three-week assay period for the A.O.A.C. ration, but decreased for ration No. 13. This increase for the A.O.A.C. ration is equivalent to approximately 48 per cent of the average initial index of  $0.90+$  (for day-old chicks), while the decrease for ration No. 13 is equivalent to approximately 24 per cent of this initial index. Although this de-

\* In all experiments, chicks that showed abnormality or disease not related to vitamin D deficiency were discarded.

<sup>4</sup> For explanation of the weighting effect of the group ashing procedure, see discussion in Part I, *This Journal*, 24, 190 (1941).

crease in gizzard erosion in chicks on ration No. 13 is coincident with greater uniformity of bone ash results, the observations are too limited to conclude that a general relationship exists.

TABLE 2.—*Comparison of rations with respect to gizzard erosion in chicks 3 weeks old*

RATION	NO. OF CHICKS	AV. INDEX <sup>a</sup> OF GIZZARD EROSION PER CHICK
A.O.A.C.	225	1.33 +
No. 13	220	0.68 +

<sup>a</sup> Scale used:	Index	Gizzard Erosion
	0	None
	1+	Extremely low to low
	2+	Medium to high
	3+	Very high to extreme

The increase in the total spread in per cent bone ash between the 0 and 15 unit levels of vitamin D intake, as shown in Table 3 and Figure 1,

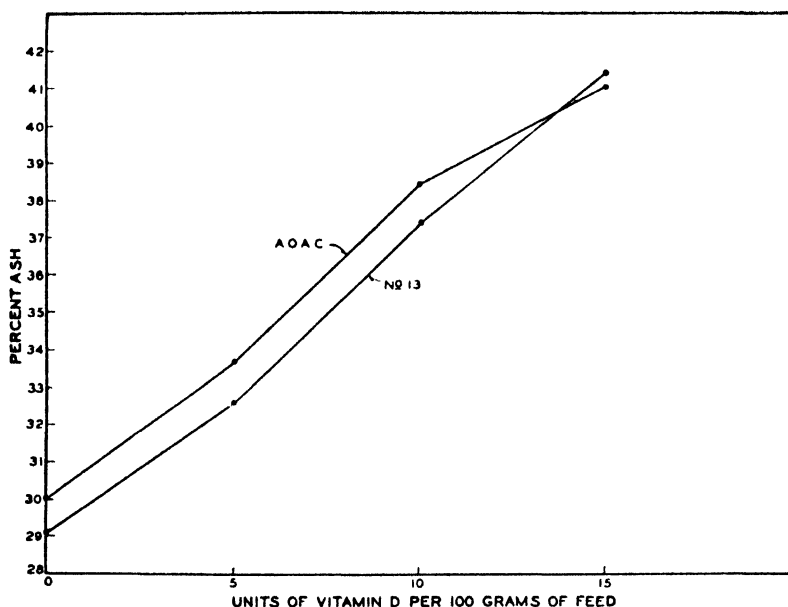


FIG. 1.—COMPARISON OF RATIONS WITH RESPECT TO TOTAL SPREAD IN PER CENT BONE ASH OF CHICKS BETWEEN 0 AND 15 UNIT LEVELS OF VITAMIN D.

indicates that a greater sensitivity of the method was obtained with ration No. 13 than with the A.O.A.C. ration. Also, the variations in per cent bone ash between repeated experiments were smaller, as a whole, and more constant. That is, a more uniform response to vitamin D intake occurred between repeated experiments.

The significance of the mean difference between the per cent bone ash for chicks weighing more than 100 grams and that for all chicks regardless

of weight is shown in Table 4. The mean difference obtained with ration No. 13, less than one-half that obtained with the A.O.A.C. ration, indicates that all chicks, regardless of weight, responded more uniformly to the experimental ration. In this respect, therefore, ration No. 13 appears to be better than the A.O.A.C. ration.

TABLE 3.—*Comparison of rations with respect to group ash of the left tibiae of chicks weighing more than 100 grams*

	RATION							
	A.O.A.C.				NO. 13			
Units of Vit. D per 100 grams of ration	0	5	10	15	0	5	10	15
No. of experi- ments	9	5	5	9	9	5	5	9
No. of chicks	115	88	91	151	105	83	85	162
Per cent bone ash								
Av.	30.03	33.67	38.47	41.04	29.11	32.57	37.38	41.38
St. Dev. <sup>a</sup>	1.03	1.48	2.37	1.57	1.28	1.32	1.51	1.51
Total spread in per cent bone ash between 0 and 15 unit levels			11.01				12.27	
							(or 11 % greater than A.O.A.C.)	

<sup>a</sup> This standard deviation shows the variability of per cent bone ash between experiments (not within each experiment as would be shown by an average standard deviation) and is given by the formula:

$$\sigma = \sqrt{\frac{nSX^2 - (SX)^2}{n(n-1)}}, \text{ where } n = \text{number of experiments.}$$

TABLE 4.—*Comparison of rations with respect to significance of mean difference between per cent bone ash for chicks weighing more than 100 grams and for all chicks regardless of weight*

RATION	CHICKS USED	NO. OF CHICKS	MEAN DIFFERENCE IN PER CENT BONE ASH	<i>t</i> VALUE <sup>a</sup>	<i>P</i> <sup>a</sup>
A.O.A.C.	> 100 g.	418			
A.O.A.C.	All	569	0.585	6.63	<0.001
No. 13	> 100 g.	423			
No. 13	All	568	0.254	2.45	0.022

$$t = \frac{M_1 - M_2}{\sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}}, \text{ where } M_1 \text{ and } M_2 \text{ are the means, } \sigma_1 \text{ and } \sigma_2 \text{ are the standard deviations, and } N_1 \text{ and } N_2 \text{ are the number of chicks.}$$

<sup>a</sup> Probability showing significance of difference. *P* = 0.05 is usually considered as indicating a significant difference and *P* = 0.01, a highly significant difference. This probability is obtained from a table of "*t*" values, such as given in almost any statistical text.

The significance of the mean difference in the standard deviations of the per cent bone ash for all chicks regardless of weight for the two rations is shown in Table 5. Although the average standard deviation for ration No.

13 is about 10 per cent lower than that for the A.O.A.C. ration, this difference is scarcely significant, as is shown by the probability of 0.068.

TABLE 5.—*Comparison of rations with respect to significance of mean difference between standard deviations of per cent bone ash of chicks*

RATION	NO. OF GROUPS	AV. NO. OF CHICKS PER GROUP	AV. ST. <sup>9</sup> DEV. OF PER CENT BONE ASH PER GROUP	MEAN DIFFERENCE	<i>t</i> VALUE <sup>10</sup>	<i>P</i> <sup>8</sup>
A.O.A.C.	26	22	3.19	0.32	1.93	0.068
No. 13	26	22	2.87			

<sup>9</sup> The average standard deviation =  $\sqrt{\frac{\sigma_1^2 + \sigma_2^2 + \dots + \sigma_n^2}{n}}$ , where "n" variances are averaged

without weighting by the number of chicks in each group, and  $\sigma^2 = \frac{NSX^2 - (SX)^2}{N(N-1)}$  for "N" chicks. (The variance is the square of the standard deviation.)

$$^{10} t = \frac{\sigma_1 - \sigma_2}{\sqrt{\frac{\sigma_1^2}{2N} + \frac{\sigma_2^2}{2N}}}$$

Comparisons of the results of the individual and group (calculated) ashing procedures are given in Table 6 and Figure 2. The two respective lines for each ration are approximately parallel until maximum calcifica-

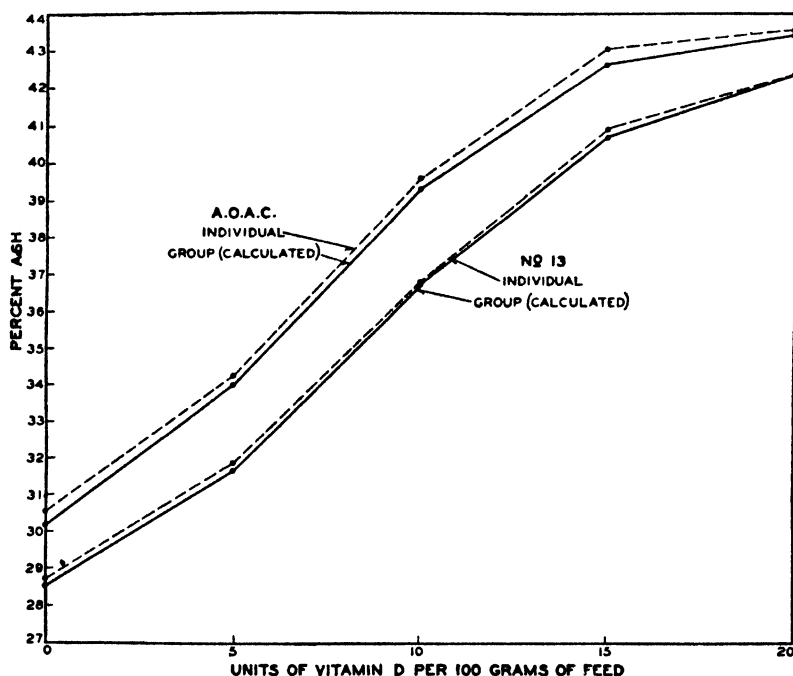


FIG. 2.—COMPARISON OF RATIONS WITH RESPECT TO INDIVIDUAL AND GROUP (CALCULATED) ASHING PROCEDURES INVOLVING ALL CHICKS REGARDLESS OF WEIGHT.



TABLE 6.—Comparison of rations with respect to body weight of chicks, per cent bone ash, and regression of per cent bone ash on body weight

UNITS OF VIT. D PER 100 G. OF RA- TION	NO. OF GROUPS PER RATION	NO. OF CHICKS	BODY WEIGHT (GRAMS)			PER CENT BONE ASH						REGRESSION OF PER CENT BONE ASH ON BODY WEIGHT <sup>11</sup>						
			RATION			RATION						SLOPE AND ERROR OF SLOPE	STANDARD ERROR OF ESTIMATE	RATON	A.O.A.C. NO. 13			
			A.O.A.C.			NO. 13			A.O.A.C.							NO. 13		
			AV. ST. DEV. <sup>9</sup>	AV. ST. DEV. <sup>9</sup>	AV. ST. DEV. <sup>9</sup>	GROUP AV. (CALC.)	INDIV ST. DEV. <sup>9</sup>	AV. ST. DEV. <sup>9</sup>	GROUP AV. (CALC.)	INDIV ST. DEV. <sup>9</sup>	AV. ST. DEV. <sup>9</sup>					GROUP AV. (CALC.)	INDIV ST. DEV. <sup>9</sup>	AV. ST. DEV. <sup>9</sup>
0	4	87	106	17	107	17	30.20	30.55	2.77	28.53	28.73	2.20	-0.0663 ± 0.0148	-0.0256 ± 0.0144	2.38	2.24		
5	4	87	120	23	120	23	34.00	34.24	3.47	31.65	31.86	2.78	-0.0313 ± 0.0160	-0.0044 ± 0.0135	3.52	2.86		
10	4	82	142	35	138	30	39.32	39.62	4.04	36.72	36.80	3.52	-0.0149 ± 0.0134	+0.0096 ± 0.0130	4.20	3.50		
15	4	83	79	143	42	144	34	42.57	43.01	3.07	40.68	40.89	3.23	-0.0283 ± 0.0070	-0.0137 ± 0.0108	2.84	3.30	
20	4	82	80	149	36	147	32	43.38	43.49	2.29	42.32	42.30	2.55	-0.0090 ± 0.0073	+0.0059 ± 0.0091	2.30	2.68	

<sup>11</sup> In brief, in the equations of these lines,  $X$  denotes the body weight of chick and  $Y$ , the per cent bone ash. The equation of a line is  $Y = a + bX$ , where " $b$ ," the slope, is here calculated by means of the method of least squares,

$$b = \frac{NSXY - SXSX}{NSX^2 - (SX)^2},$$

and " $a$ " is the "y" intercept:

$$a = \bar{Y} - b\bar{X}, \text{ where } \bar{Y} = \text{mean of } Y, \text{ and } \bar{X} = \text{mean of } X.$$

The standard error of estimate showing the scatter about the line is calculated from the formula:

$$\sigma_{\text{est } Y} = \sqrt{\sigma_y^2 - b^2\sigma_x^2}, \text{ where } \sigma_y^2 \text{ is variance of } Y, \text{ and } \sigma_x^2 \text{ is variance of } X.$$

tion is approached, at which point they tend to coincide. There is less difference in the results of the two ashing procedures for ration No. 13 than in those for the A.O.A.C. ration, which indicates that extreme variations among individual chicks are not so great for ration No. 13. This point is confirmed in Table 6 by the fact that the average standard deviation of the individual ash percentages are, as a whole, slightly less for ration No. 13. Also, as shown in Table 6 and Figure 2, a larger total spread in per cent bone ash was obtained with the experimental ration. However, this difference between the two rations, as reflected by the total spread in per cent bone ash, is not so pronounced for these four experiments as it is for all nine experiments discussed under Table 3 and Figure 1.

TABLE 7.—*Comparison of rations with respect to significance of difference in per cent bone ash of chicks between adjacent levels of vitamin D (Each level involves 4 groups of approximately 21 chicks each per ration.)*

ADJACENT LEVELS OF VITAMIN D (UNITS PER 100 G. OF RATION)	AV. DIFFERENCE IN PER CENT BONE ASH		t VALUE <sup>12</sup> FOR AV. DIFFERENCE		P FOR AV. DIFFERENCE <sup>8</sup>	
	RATION		RATION		RATION	
	A.O.A.C.	NO. 13	A.O.A.C.	NO. 13	A.O.A.C.	NO. 13
0 and 5	3.80	3.12	4.02	4.09	<0.001	<0.001
5 and 10	5.32	5.07	4.62	5.15	<0.001	<0.001
10 and 15	3.25	3.96	2.82	3.70	0.01	<0.001
15 and 20	0.81	1.64	0.91	1.78	0.38	0.09
Average	3.23	3.38	3.15	3.64	0.005	<0.001

$$^{12} t = \frac{M_a - M_b}{\sqrt{\frac{\sigma_a^2}{N_a} + \frac{\sigma_b^2}{N_b}}}, \text{ where } M_a - M_b \text{ is average difference in per cent bone ash, } \sigma_a \text{ and } \sigma_b \text{ are average standard deviations, and } N_a \text{ and } N_b \text{ are average number of chicks.}$$

Although the average difference in per cent bone ash for adjacent levels of vitamin D intake (Table 7) is in some instances less for ration No. 13, the probability of overlapping is less for this ration because of lower individual variations (as shown by the standard deviations). This is indicated by the larger "t values" for ration No. 13 shown in Table 7 (the larger the "t value" for a given number of degrees of freedom, the smaller the probability associated with it). However, the differences in per cent bone ash for adjacent levels of vitamin D intake are definitely significant for both rations except when maximum calcification is approached, as is shown by the small increase in per cent bone ash between the 15 and 20 unit levels of vitamin D intake.

The comparisons of the regressions of per cent bone ash on chick weight for the various levels of vitamin D intake are shown in Table 6 and Figure 3. The lines showing the relationship of per cent bone ash to body weight of the chicks were fitted by the method of least squares<sup>11</sup> to data for each level of vitamin D fed. The regression coefficients (or slopes of the lines, as given in Table 6) for ration No. 13 are in all cases less than those for

the A.O.A.C. ration. In other words, the weight of the chick had less effect on the per cent bone ash for ration No. 13 than on that for the

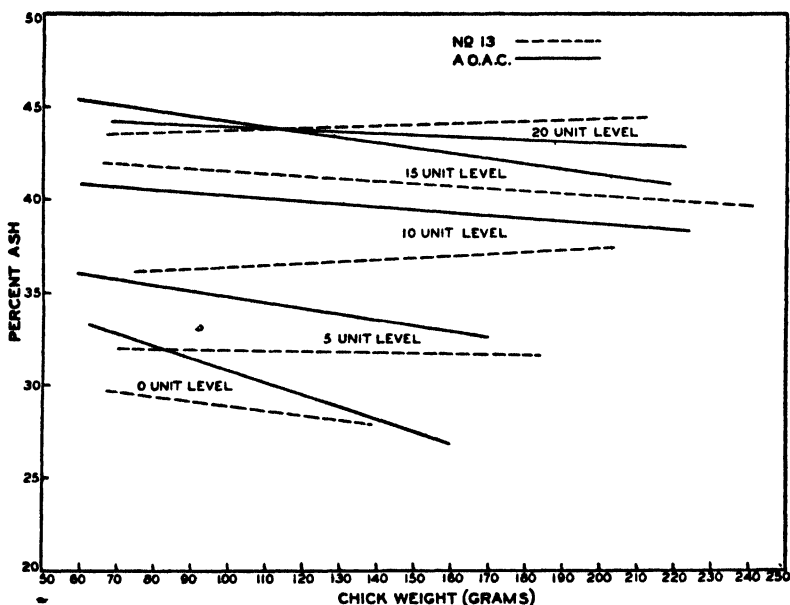


FIG. 3.—COMPARISON OF RATIONS WITH RESPECT TO RELATIONSHIP OF PER CENT BONE ASH TO BODY WEIGHT OF CHICKS.

A.O.A.C. ration. This can also be seen by comparing the slopes of the lines in Figure 3.

#### SUMMARY

A modified basal ration for use in the chick method for the assay of vitamin D has been subjected to a critical study. By means of feeding experiments, this basal ration has been compared with the present A.O.A.C. basal ration and has been found to have the following advantages:

- (1) The total spread in per cent bone ash between the minimum and maximum levels of vitamin D intake is greater, resulting in an increased sensitivity of the method.
- (2) The reproducibility of response to given levels of vitamin D intake as measured by per cent bone ash in repeated experiments is more satisfactory.
- (3) The mean difference between the per cent bone ash for chicks weighing more than 100 grams and that for all chicks regardless of weight is significantly decreased.
- (4) The average standard deviation of the per cent bone ash for all chicks regardless of weight is decreased.
- (5) The variation between the individual and group ashing procedures is reduced.
- (6) The probability of overlapping of the bone ash percentages of adjacent levels of vitamin D intake is decreased.
- (7) The influence of the body weight of the chicks on the per cent bone ash is less marked.

## ASH DETERMINATIONS IN FOODS WITH AN ALKALINE BALANCE

### II. DECOMPOSITION OF ALKALI, CALCIUM, AND MAGNESIUM CARBONATES

By H. J. WICHMANN (Food Division,\* U. S. Food and Drug  
Administration, Federal Security Agency, Washington, D. C.)

In a previous paper (1) it was shown that after normal ashes had been carbonated with ammonium carbonate solutions, the water evaporated and the residues heated at 260°–300° C., they were distinctly heavier than the original material. However, carbonated ashes reheated at 525°–600° C. (temperatures at which the normal ashes were produced) yielded results very close to the original normal ashes. The second finding confirmed earlier observations, but the first appeared new and perhaps indicated that earlier investigators had been heating their carbonated ashes at too high temperatures. These results were interpreted in the earlier publication as being due to carbonation of oxides produced during the first ashing, and to decomposition of magnesium carbonate and, to some extent, of calcium carbonate on reheating the carbonated ashes at 525°–600° C. The writer's idea was to produce a fully carbonated ash at a temperature so high that neither water nor bicarbonates could continue to exist, but not high enough to cause decomposition of "susceptible" carbonates. At that time it was not considered that phosphates were involved in any carbonation reactions involving weight increases. The next step in the investigation, therefore, was to determine definitely just what compounds contributed to the results found, and also the manner and degree. This project required some fundamental studies on the various compounds present in ashes. Optimum temperatures and the chemical behavior of ash constituents at these temperatures were also involved. It was hoped that the real meaning of the term "ash" would thus become clear and that rational ash methods would result. The first ingredients of alkaline plant ashes studied were alkali, calcium, and magnesium carbonates.

### DECOMPOSITION OF ALKALI CARBONATES

Potassium compounds predominate in the ashes of plants, but sodium is more abundant in ashes of animal origin. Therefore it is important to know whether the carbonates of these two common alkali metals decompose into oxides at ordinary ashing temperatures of 500°–600° C. under the influence of heat or hot carbon, or of both.

Mellor (2) reports that sodium and potassium carbonates do not lose weight when heated to redness in an atmosphere of carbon dioxide, but do decompose to some extent in hydrogen, nitrogen, or carbon dioxide-

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\* W. B. White, Chief.

free air. R. A. Osborn (private communication) found that sodium carbonate prepared from the bicarbonate at temperatures above  $300^{\circ}\text{C}$ . was always hygroscopic owing to the formation of slight amounts of the oxide. Smith and Croad (3) demonstrated, both by weight losses and by the absorption of carbon dioxide, that sodium carbonate heated above  $300^{\circ}\text{C}$ . suffers a small but appreciable decomposition, which is proportional to the time of heating and the temperature. Alfend (4), in reporting as Associate Referee on Eggs, quotes some unpublished investigations of Clarke and Mitchell, to the effect that there is apparently no loss when sodium or potassium carbonates are ignited with organic matter at  $500^{\circ}\text{C}$ . and leached, and that there is little if any loss if they are then ignited at  $650^{\circ}\text{C}$ . It is further claimed by Clarke and Mitchell (4) that sodium carbonate remains practically unchanged on ignition at  $700^{\circ}\text{C}$ . but volatilizes appreciably at  $900^{\circ}\text{C}$ . and that potassium carbonate volatilizes appreciably at  $700^{\circ}\text{C}$ . and almost completely at  $900^{\circ}\text{C}$ . The writer confirms these conclusions, and at this time presents additional evidence on the decomposition of potassium carbonate, an ingredient of prime importance in all alkaline plant ashes.

About 100 mg. of pure, dry potassium carbonate in a platinum dish was dissolved in distilled water, the water was evaporated on a steam bath, and the residues and dish were then heated in a temperature-controlled muffle for 15 minute intervals at increasing temperatures starting at  $200^{\circ}\text{C}$ . After the heating, the dish was covered with a duraluminum cover (1), cooled in an aluminum desiccator, and weighed. The weight changes found are represented by the curve in Chart 1.

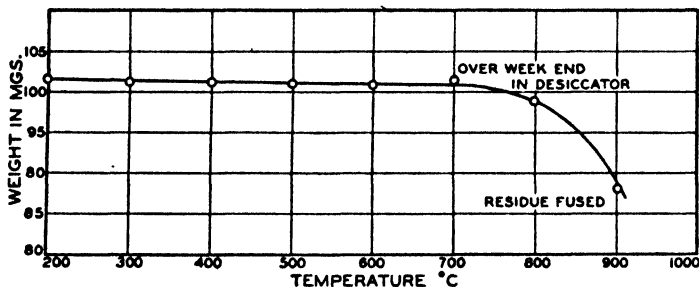


CHART 1.—DECOMPOSITION CURVE OF  $\text{K}_2\text{CO}_3$ . HEATING INTERVAL 15 MINUTES.

The loss in weight of potassium carbonate between  $200^{\circ}$  and  $700^{\circ}\text{C}$ . is just barely perceptible, and seems to correspond closely to the rate of decomposition of sodium carbonate reported in the literature. It cannot, however, account for the ash data in Table 2 of the writer's previous paper (1). The decided break in the curve above  $700^{\circ}\text{C}$ . confirms the observations of Clarke and Mitchell.

An effort was made to determine whether the loss of weight at  $700^{\circ}\text{C}$ .

was due to decomposition of the carbonate to the oxide or to actual volatilization of potassium compounds. About 100 mg. of potassium carbonate was dried at 250° C., weighed in a covered platinum dish, and then heated, uncovered, for 2 hours at 700° C. The dish was then covered, cooled in a desiccator, and weighed. The initial and final weights were 112.9 and 108.0 mg., respectively, showing a loss of about 4 per cent. After carbonation with carbon dioxide water, drying at 250° C. for 30 minutes, cooling, and weighing (dish covered), the weight was 109.6 mg. This result indicates that the residue contained very little potassium oxide and that actually 3.3 mg. of potassium carbonate had disappeared. In another experiment in which a temperature of 900° C. was used for 30 minutes, the loss in weight was 16 per cent. Vapors were seen rising from the dish when it was removed from the furnace. Carbonation resulted in 1 mg. increase in weight. Again, the residue consisted almost entirely of the carbonate.

These experiments do not show whether the potassium carbonate volatilizes as such at 700° C. or above, or first decomposes to the oxide, which then volatilizes rapidly. But whatever the mechanism of volatilization, studies on changes in composition of other carbonates can hardly be complicated to any appreciable extent by the simultaneous decomposition of coexisting alkali carbonates at temperatures below 700° C.

#### DECOMPOSITION OF CALCIUM CARBONATE

The dissociation temperature of calcium carbonate at 760 mm. pressure of carbon dioxide, as determined by various authorities, varies from 812° to 920° C. Mellor (5) believes that the value 890° C. is nearest the truth. During any ashing at 500°–600° C. the carbon dioxide produced by burning organic matter, unless removed, would lie like a blanket over the ash and hinder the decomposition of carbonates therein. But this carbon dioxide will leak out of the muffle more or less rapidly, the rate depending upon the construction of the furnace. In the last phase of an ashing, there is likely to be little excess carbon dioxide in the air inside the muffle and conditions are probably more or less similar to those present when unconfined ashes are heated in ordinary air. When the carbon dioxide is removed rapidly, any calcium or magnesium carbonate in the ash will naturally decompose at lower temperatures.

Clarke and Mitchell (4) have studied some of the reactions of calcium carbonate in air in connection with ash determinations. They comment as follows: "Calcium carbonate, in the presence of organic matter, undergoes little or no change on ignition at 500° C.; it decomposes gradually though irregularly, and reaches minimum weight on ignition at 700° C.; and it does not undergo further change on ignition at 900° C."

Decomposition studies similar to those with potassium carbonate, within the temperature range 200°–700° C., were begun on calcium carbonate. The work was facilitated by the installation of a new muffle and by a

temperature-control device that was believed to be effective in controlling the temperature of ash dishes (placed on an elevated silica board close to the thermocouple) within  $\pm 5^\circ \text{C}$ . Calcium acetate prepared from known quantities of analytical-grade calcite was mixed with ashless sucrose in a platinum dish, the mixture was ashed at  $500^\circ \text{C}$ ., and the ash was weighed in the covered dishes previously described (1). Water was added to the contents of the dish and evaporated off, and the residue was then heated for 15 minute intervals at increasing temperatures starting at  $250^\circ \text{C}$ . The water was added to simulate the usual ashing practice, and the 15 minute interval was chosen because that seemed to be the time required for the establishment of equilibrium in the ash determinations recorded

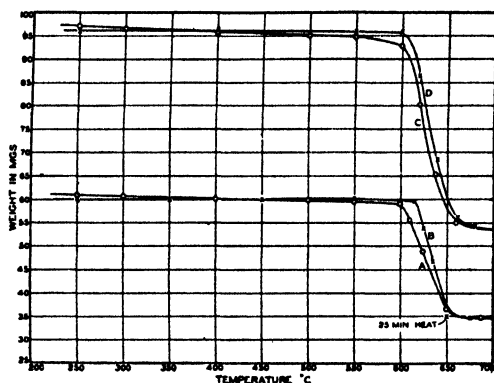


CHART 2.—DECOMPOSITION CURVES OF  $\text{CaCO}_3$ .

A.—Mixed  $\text{CaO}$  and  $\text{CaCO}_3$  carbonated with water saturated with  $\text{CO}_2$  and evaporated, and residue heated for 15 minute intervals.

B.—Calcium acetate mixed with sugar ashed to  $\text{CaCO}_3$  at  $500^\circ \text{C}$ ., water added and evaporated, and residue heated for 15 minute intervals. Weight of ash 60.2 mg.

C.— $\text{CaO}$  and  $\text{CaCO}_3$  carbonated with  $(\text{NH}_4)_2\text{CO}_3$  solution and evaporated, and residue heated for 15 minute intervals.

D.—Calcium acetate mixed with sugar, ashed to  $\text{CaCO}_3$  at  $500^\circ \text{C}$ ., water added, and evaporated, and residue heated for 15 minute intervals up to  $680^\circ \text{C}$ . Weight of ash 95.8 mg.

in Table 2 of the previous paper (1). After most of the calcium carbonate had been converted to calcium oxide at  $700^\circ \text{C}$ ., in some experiments it was reconverted to the carbonate with ammonium carbonate solution and in others with cold water saturated with carbon dioxide from a pressure bottle. Some of the data obtained are shown in the curves of Chart 2.

These curves indicate that there is little decomposition of amorphous calcium carbonate in an air-filled muffle until a temperature of  $600^\circ \text{C}$ . is reached (slightly above usual ashing temperatures). Sudden decomposition then follows, and the reaction is almost complete at  $650^\circ \text{C}$ ., at least it

was under the conditions of these experiments. Carbonation with either ammonium carbonate solutions or carbon dioxide water produced more finely grained residues, which decomposed at slightly lower temperatures than did the original carbonates. These experiments confirm the statements of Clarke and Mitchell relative to the decomposition of calcium carbonate at 500° and 700° C., and they also show what happens to calcium carbonate at intervening temperatures.

An unexpected development is shown in the crossing of the curves at 400°–450° C. Mellor (6) quotes R. Fresenius as authority for the statement that ammonium carbonate is retained tenaciously by precipitated calcium carbonate. However, a similar crossing is noted after carbonation with carbon dioxide water. Whether the small weight increase after carbonation and reheating at temperatures below 450° C. is due to retention of water or ammonium carbonate, or to undecomposed calcium bicarbonate remains uncertain, but in any event it cannot account for the much larger weight increases of the total ash after carbonation shown in the previous publication (1).

#### DECOMPOSITION OF MAGNESIUM CARBONATE

Mellor (7) gives a resumé of the available information on the action of heat on magnesium carbonate and states that the conclusions drawn by different observers are not always in agreement. Magnesium carbonate is said to lose carbon dioxide from temperatures of 200° C. to almost 600° C., depending on whether light, crystalline, or heavy carbonate or magnesite is heated. Brill (8) heated magnesium carbonate said to contain  $\frac{1}{2}$  molecule of water for 10 minute intervals in carbon dioxide at one atmosphere pressure at 200°–600° C. He found a series of breaks in the curve and believed they indicated the formation of a series of basic carbonates. Clarke and Mitchell (4) studied the heating of magnesium carbonate in ashing procedures and comment as follows: "Magnesium carbonate, in the presence of organic matter, undergoes decomposition, but does not reach uniform weight on ignition at 500° C; it reaches minimum weight on ignition at 700° C; and it does not undergo further change on ignition at 900° C." To determine what happens to magnesium carbonate at other temperatures, the writer made decomposition curves similar to those made for potassium and calcium carbonates.

Some pure crystalline metallic magnesium, obtained from the Dow Chemical Co., Midland Mich., was dissolved in dilute glacial acetic acid, and the acetate was then ashed at 500°–600° C. The weight of the theoretical anhydrous magnesium carbonate was calculated in some experiments from the weight of the metallic magnesium taken and in others from the weight of the ignited magnesium oxide. The magnesium oxide obtained in these experiments dissolved completely in 10 ml. of 10 per cent ammonium carbonate solution, or in 50–75 ml. of water saturated



with carbon dioxide from a pressure bottle, on warming on the steam bath. After evaporation of about half of the solution, precipitates again appeared. The residues were heated for 15 minute intervals at varying temperatures at and above 200° C. The results (Chart 3) show that any magnesium, in excess of that combined as the phosphate or sulfate, must exist in an ash mostly as the oxide, at ashing temperatures of 500°–600° C. These findings are directly the opposite of those shown in the previous calcium experiments. The greatest rate of loss of carbon dioxide occurs between 350° and 400° C.; beyond 400° C. the remnant of carbon dioxide is removed very slowly. At 200° C. the residue weighs more than it

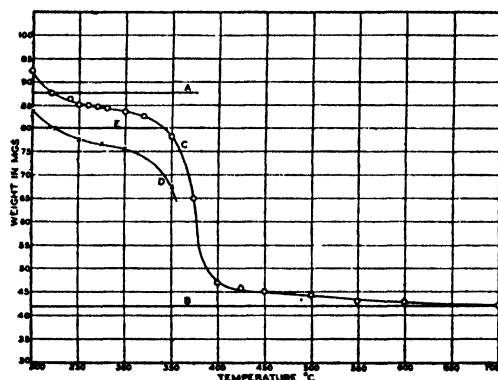


CHART 3.—DECOMPOSITION CURVES OF  $\text{MgCO}_3$ .

- A.—Weight of  $\text{MgCO}_3$  calculated from weight of  $\text{MgO}$  ignited at 600° C.
- B.—Weight of  $\text{MgO}$  ignited at 600° C.
- C.—Magnesium acetate ashed to  $\text{MgO}$  at 600° C., carbonated with  $(\text{NH}_4)_2\text{CO}_3$  solution, water evaporated, and residue heated for 15 minute intervals.
- D.—Magnesium acetate ashed to  $\text{MgO}$  at 500° C., ignited at 800° C., carbonated with water saturated with  $\text{CO}_2$ , and evaporated, and residue heated for 15 minute intervals.
- E.—Weight of  $\text{MgCO}_3$  calculated from 38.3 mg. of  $\text{MgO}$  ignited at 800° C.

should, on the assumption that it is anhydrous magnesium carbonate, but at 225° C. it appears to be that compound. Above 225° C. there is a gradually increasing loss of weight, with a sudden drop in the curve at 350° C. These characteristics of magnesium carbonate are very illuminating when considered in connection with the previously noted weight changes of carbonated ashes of agricultural products upon reheating at 300° C. and also in connection with the original temperature of the ash determination. The carbonate is fairly stable at 225°–300° C., but at 500° C. the oxide is the stable compound. The writer does not know whether phosphates in food products combine preferentially with calcium or with magnesium. If calcium is preferred by the phosphate ion, there might be sufficient residual magnesium existing as the oxide in plant ashes

to account partially or even entirely for the weight changes exhibited by carbonated ashes. This point should be investigated further.

Additional work was done to ascertain the significance of the "crossing" at 225° C. and of the region between 225° and 350° C., where the decomposition of magnesium carbonate seems to be gradual. As it had been suggested to the writer that 15 minute heating intervals might not be sufficiently long to establish constant weights, magnesium carbonate, prepared by carbonating magnesium oxide as before, was heated for consecutive periods of 15 minutes at 225° C. The difference in weight between the first and second heating was very small, and that between the second

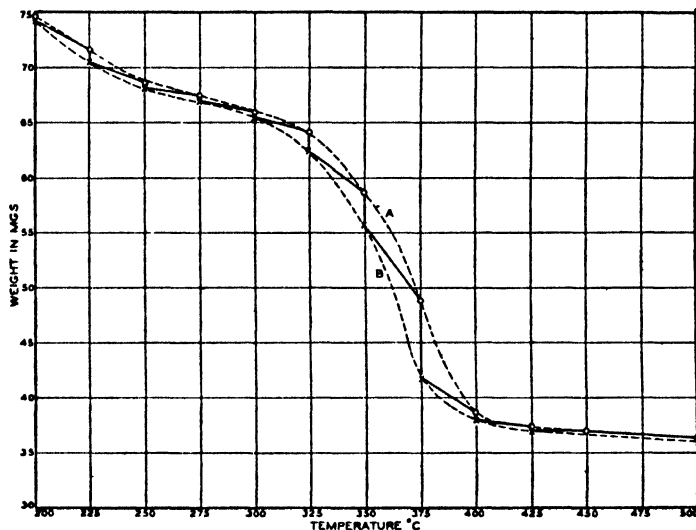


CHART 4.—DECOMPOSITION CURVE OF  $\text{MgCO}_3$  (SOLID LINE).

A.—Curve connecting points representing weights of residue after first 15 min heating.

B.—Curve connecting points representing weights of residue after second 15 min heating.

and third was still smaller, and all three points were close to the theoretical weight of anhydrous magnesium carbonate. Therefore the weight of precipitated magnesium carbonate after being heated for 15 minutes at 225° C. seems to represent an equilibrium point that closely approaches that of anhydrous magnesium carbonate. The carbonated magnesium oxide was also heated for two consecutive periods of 15 minutes each at increasing temperatures, starting at 200° C. The results are given in Chart 4. The decomposition curve has now been converted into a series of broken lines. Two smooth curves, approximately parallel to each other and very similar to the curves in Chart 3, are formed by joining the points representing the weights of the residues after the first 15 minute heating and likewise those representing the weights after heating for 30

minutes. The loss in weight between two consecutive heatings at the same temperature is generally smaller than it is when the temperature is raised 25° C., and it becomes practically insignificant when the rate of decomposition is slow. But at the temperatures at which the rate of decomposition of magnesium carbonate becomes rapid, time has significance. This is shown clearly at 350° and 375° C. Above 400° C. the difference between two heating intervals again becomes small.

The staggered curve indicates that there are some significant breaks or equilibrium points in the decomposition of magnesium carbonate to magnesium oxide above 225° C., and possibly the formation of a series of basic carbonates as first postulated by Brill (8). At temperatures below the crossing at 225° C., the weight of the residue is too high, which may indicate the retention of a small quantity of water or the incomplete elimination of bicarbonate. The crossing at 225° C., therefore, seems to be significant, and it might establish this temperature as optimum for heating carbonated ashes containing magnesium carbonate, if potassium carbonate presents no complication. It is therefore necessary to determine whether alkali carbonates have any effect on the decomposition of magnesium and calcium carbonates.

#### DECOMPOSITION OF MAGNESIUM CARBONATE IN PRESENCE OF POTASSIUM CARBONATE

The results of the decomposition experiments made with magnesium and potassium carbonates are shown in Curves 1 to 7 in Charts 5 and 6. Curves 1 and 2 represent the decomposition curves of 34.67 mg. of anhydrous magnesium carbonate, respectively, in air and in an atmosphere of carbon dioxide obtained by bubbling a stream of the dried gas into the furnace. Curves 3 to 7 represent data obtained with the same quantity of magnesium carbonate as was used before but with increasing quantities of potassium carbonate, the quantities and ratios to the magnesium carbonate being indicated in the figures.

Bubbling carbon dioxide into the furnace during the heating period resulted in a very moderate shifting of the curves upward and to the right. The partial pressure of the carbon dioxide was probably much below one atmosphere owing to difficulties of manipulation and leaks in the furnace, but the retarding effect of the carbon dioxide on the decomposition of magnesium carbonate is very apparent, the crossing being now at 300° C. At 500° C., however, the effect of the carbon dioxide has been lost and Curves 1 and 2 coincide. There seems to be no advantage in heating carbonated ashes in an atmosphere of carbon dioxide because of the difficulties of manipulation with ordinary muffle furnaces and the fact that the general shape of the decomposition curve of magnesium carbonate is not essentially altered, but merely shifted somewhat in position. Optimum temperature would still demand attention.

As the ratio of potassium carbonate to magnesium carbonate increases, greater resistance to decomposition in the lower temperature ranges is exhibited by the magnesium carbonate. There is a progressive shift of the crossing first to 300° C., then to 325° C., and finally to about 335° C. Increasing the ratio of potassium to the magnesium carbonate somewhat

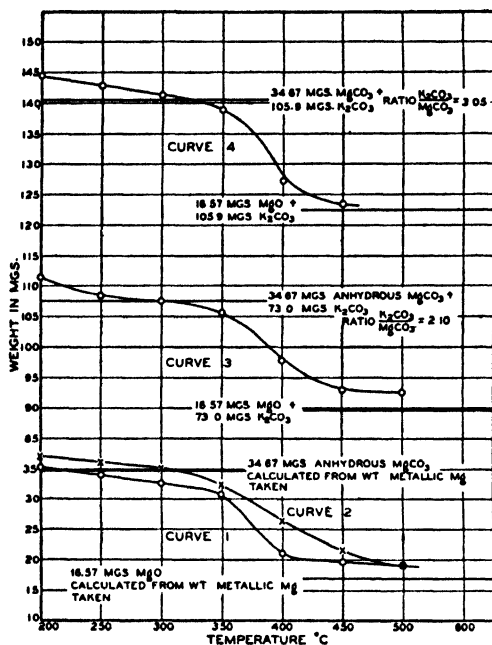


CHART 5.—DECOMPOSITION CURVES OF  $MgCO_3$  IN AIR, IN ATMOSPHERE OF  $CO_2$ , AND IN PRESENCE OF  $K_2CO_3$ . HEATING INTERVALS 15 MINUTES.

Curve 1.—Decomposition curve of  $MgCO_3$  heated in air.

Curve 2.—Decomposition curve of  $MgCO_3$  heated in atmosphere containing  $CO_2$ .

Curve 3.—Decomposition curve of a mixture of 34.67 mg.  $MgCO_3$  and 73.0 mg.  $K_2CO_3$ .

Curve 4.—Decomposition curve of a mixture of 34.67 mg.  $MgCO_3$  and 105.9 mg.  $K_2CO_3$ .

slowed down the rate of decomposition between 350° and 450° C., but at 450° C. the decomposition was practically complete. The weights at temperatures between 200° C. and the crossing point are consistently greater than the sum of the weights of dry potassium carbonate and the theoretical anhydrous magnesium carbonate. This extra weight and the increased resistance towards heat in this temperature range may mean the formation of double carbonates with possibly resistant water of crystallization. Mellor (9) mentions double carbonates of potassium and magnesium with either the formula  $K_2Mg(CO_3)_2 \cdot 4H_2O$  or  $Mg(KCO_3) \cdot HCO_3 \cdot 4H_2O$ .

On the other hand, increased heat resistance beyond the crossing may be due to some blanketing or mass-action effect of the solid potassium carbonate similar to that of carbon dioxide in the atmosphere above the solid magnesium carbonate. Some work was done in an effort to explain these two possibly simultaneous effects, but a description of this part of the work hardly seems appropriate in a paper devoted to ash methods.

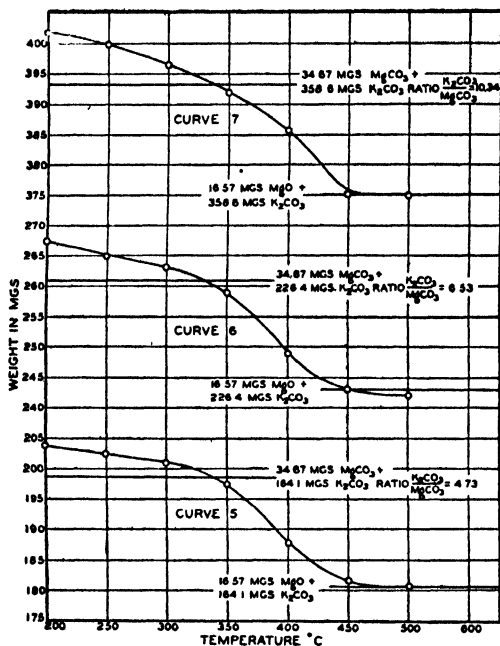


CHART 6.—DECOMPOSITION CURVES OF MIXTURE OF  $\text{MgCO}_3$  WITH  $\text{K}_2\text{CO}_3$ . HEATING INTERVAL 15 minutes.

Curve 5.—Decomposition curve of a mixture of 34.67 mg.  $\text{MgCO}_3$  and 164.1 mg.  $\text{K}_2\text{CO}_3$ .

Curve 6.—Decomposition curve of a mixture of 34.67 mg.  $\text{MgCO}_3$  and 226.4 mg.  $\text{K}_2\text{CO}_3$ .

Curve 7.—Decomposition curve of a mixture of 34.67 mg.  $\text{MgCO}_3$  and 358.6 mg.  $\text{K}_2\text{CO}_3$ .

It is evident from the data given that 225° C. (crossing point for magnesium carbonate heated by itself) is not the optimum temperature for heating carbonated ashes containing both magnesium and excess potassium carbonate, and the latter is a large constituent of many plant ashes. If any given ash should contain an excess of both potassium and magnesium carbonate over phosphate requirements (or sulfate or chloride combinations), it might be necessary to adjust the optimum heating temperature to it, and quite likely it would vary from the temperature required for other products. This point will be tested later by the production of

decomposition curves of ashes of typical products differing in mineral composition.

### DECOMPOSITION OF CALCIUM CARBONATE IN PRESENCE OF POTASSIUM CARBONATE

Experiments similar to those with magnesium just described were made on the decomposition of calcium carbonate in the presence of carbon dioxide gas and potassium carbonate. The data are given in the curves of Chart 7 and should be self-explanatory in the light of previous discus-

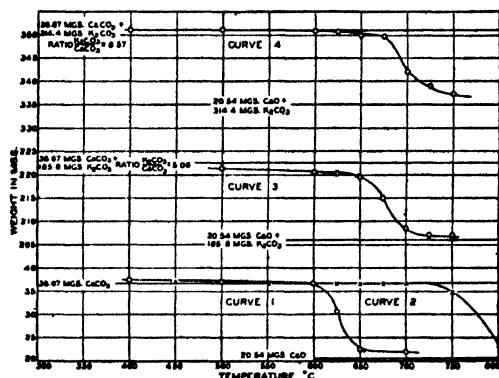


CHART 7.—DECOMPOSITION CURVES OF  $\text{CaCO}_3$  HEATED IN AIR, IN ATMOSPHERE CONTAINING  $\text{CO}_2$ , AND IN PRESENCE OF  $\text{K}_2\text{CO}_3$ .

HEATING INTERVALS 15 MINUTES.

Curve 1.—Decomposition curve of  $\text{CaCO}_3$  heated in air.

Curve 2.—Decomposition curve of  $\text{CaCO}_3$  heated in atmosphere containing  $\text{CO}_2$ .

Curve 3.—Decomposition curve of a mixture of 36.67 mg.  $\text{CaCO}_3$  and 185.8 mg. of  $\text{K}_2\text{CO}_3$ . Carbonation with  $(\text{NH}_4)_2\text{CO}_3$  solution.

Curve 4.—Decomposition curve of a mixture of 36.67 mg.  $\text{CaCO}_3$  and 314.4 mg.  $\text{K}_2\text{CO}_3$ . Carbonation with  $\text{CO}_2$  water.

sion. It is evident that calcium carbonate is much more heat resistant in a partial atmosphere of carbon dioxide than is magnesium carbonate. A tight furnace that retains the carbon dioxide produced by the oxidation of organic matter will no doubt retard the loss of carbon dioxide from calcium carbonate even well above ashing temperatures.

Potassium carbonate also had the effect of retarding the decomposition of an alkaline earth carbonate. Mellor (10) lists a number of complex carbonates of potassium or sodium with calcium, strontium, and barium, that are said to break down with greater difficulty than does calcium carbonate alone. Again it is not clear whether the heat-resisting effect of potassium carbonate on calcium carbonate is due to a complex formation, to a mass action effect, or to the simultaneous action of both. Extensive study would be necessary to determine the facts on these assumptions as applied

to the decomposition of both magnesium and calcium carbonates, but this also is apart from the main objective, ash methods.

### DECOMPOSITION OF MIXED CALCIUM AND MAGNESIUM CARBONATES

The next step in this investigation was to determine whether the formation of double alkaline earth carbonates is possible under carbonating conditions. A decomposition curve was made as before with a mixture of magnesium and calcium oxides carbonated to their respective carbonates. Curve 1 in Chart 8 shows the results. There seems to be no evidence that

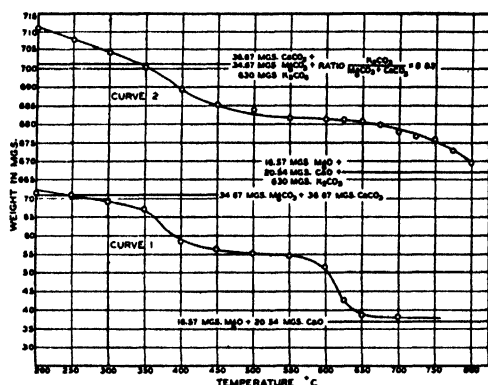


CHART 8.—DECOMPOSITION CURVES OF MIXTURES OF  $\text{MgCO}_3$  and  $\text{CaCO}_3$  AND MIXTURE OF  $\text{MgCO}_3$ ,  $\text{CaCO}_3$  and  $\text{K}_2\text{CO}_3$ . HEATING INTERVALS 15 MINUTES. CARBONATION WITH  $\text{CO}_2$  WATER.

Curve 1.—Decomposition curve of a mixture of 34.67 mg.  $\text{MgCO}_3$  and 36.67 mg.  $\text{CaCO}_3$ .

Curve 2.—Decomposition curve of a mixture of 34.67 mg.  $\text{MgCO}_3$ , 36.67 mg.  $\text{CaCO}_3$ , and 630 mg.  $\text{K}_2\text{CO}_3$ .

dolomite is formed. This curve shows two points of decomposition that correspond to the previously determined points of inflection of the separate carbonates. Mixing the carbonates merely joins two decomposition curves into one.

### DECOMPOSITION OF MIXED POTASSIUM, CALCIUM, AND MAGNESIUM CARBONATES

Curve 2 of Chart 8 represents the decomposition curve of a carbonated mixture of calcium and magnesium oxides with excess potassium carbonate. The evidence of possible complex formations between magnesium carbonate and potassium carbonate, and between calcium carbonate and potassium carbonate are again present, as shown by the differences between Curve 1 and Curve 2. As in Curve 1, there are two breaks in the single decomposition curve, and both are shaped and located as shown in

the curves of Charts 5, 6, and 7. There seems to be no evidence in Curve 2 of the formation of triple carbonates.

There is one other interesting feature in Curve 2. In Curves 3 and 4 of Chart 7 the rate of decomposition of calcium carbonate beyond 650° or 675° C. was quite rapid, but in this case it is more gradual. If the presence of potassium-magnesium carbonate and potassium-calcium carbonate complexes is assumed, the former must have been decomposed at 500° C., as indicated by the flat portion of the curve beyond that temperature, with consequent liberation of the potassium carbonate and a great increase in the ratio of that compound to calcium carbonate. The potassium-calcium carbonate complex began to decompose at 650° C. (Chart 7), but something prevented the usual rapid decomposition of the complex above that temperature, which indicates that the potassium carbonate in this experiment had a "complexing" effect on the calcium carbonate that carried the complex to about 650° C. without appreciable decomposition, and that the heat-resisting powers of the mixture above 650° C. are due mainly to a mass action effect that increases with the mass of the alkali carbonate.

#### SUMMARY AND CONCLUSIONS

(1) Potassium carbonate heated in air is decomposed only slightly below 700° C. Precipitated calcium carbonate heated in air decomposes markedly between 600° and 650° C., and magnesium carbonate between 300° and 400° C. At usual ashing temperatures, therefore, the stable compound of magnesium is the oxide, but the other two exist as the carbonates.

(2) Both magnesium and calcium carbonates are more stable under heat treatment in the presence of potassium carbonate, due to the formation of complex carbonates or mass action effect, or both.

(3) Carbonation of mixtures of calcium and magnesium oxides followed by heat treatment produces no dolomite. Adding potassium carbonate to such mixtures produces no triple alkali-alkaline-earth carbonates.

The next objective in the investigation is to make decomposition curves of typical agricultural products to determine whether calcium or magnesium carbonates are actually produced and decomposed under ordinary ashing conditions similar to those shown in Charts 2-8.

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## DETERMINATION OF POTASSIUM IN FRUIT PRODUCTS: A STUDY OF THE WILCOX COBALTINITRITE METHOD

By C. A. Wood (U. S. Food and Drug Administration, New York, N. Y.)

The A.O.A.C. method for the estimation of potassium by precipitation as the chloroplatinate from the ash of fruit products requires considerable manipulation of the sample and is quite time-consuming. Experience with the procedure is often necessary before concordant results are obtained. For example, during the sulfating treatment care should be exercised to prevent mechanical loss, and likewise much attention is needed during the evaporation of the aqueous chloroplatinate solution to small volume in the platinum dish. Local drying of this residue yields, at times, a poor precipitation of the potassium chloroplatinate.

Wilcox<sup>1</sup> has developed a method specifying a solution of the pure trisodium cobaltinitrite salt for precipitating the potassium ion. The solution under test is acidified with nitric acid and 0.01 *N* nitric acid is used for washing the precipitate in order to retard the decomposition of nitrites. In this way, stoichiometric results are obtained by one simple precipitation. The method has been tested in the presence of the cations that usually occur in significant amounts in the fruit ash. Results indicate no interference. The analyst is warned, however, against the possibility of precipitation of silica dissolved in the solution of plant ash material. Wilcox claims accuracy and reproducibility of  $\pm 0.05$  mg. of potassium in the range from 2 to 15 mg. of the metal.

The cobaltinitrite estimation has not previously been applied to the analysis of fruit products. Therefore, the writer made a number of determinations for potassium, applying the Wilcox technic to the ash of various fruits and jams that previously had been analyzed by the A.O.A.C. method. The acidified solution from the ash was allowed to stand before being filtered in order to provide for precipitation of material, such as silica, on standing. The details of the gravimetric procedure follow:

### REAGENTS

(a) *Trisodium cobaltinitrite solution*.—Prepare an aqueous solution containing 0.94 gram of the salt of reagent quality in each 5 ml. ( $K = 0.00\%$  or its equivalent). Filter before use and prepare a fresh lot of solution before each set of determinations.

(b) *Nitric acid*.—Approximately 1 *N* and 0.01 *N*.

(c) *Ethyl alcohol*.—95%.

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<sup>1</sup> *Ind. Eng. Chem.*, **9**, 136 (1937).

## DETERMINATION

Use normal or sulfated ash from the fruit or jam. Add 1 *N* HNO<sub>3</sub> to the ash in the Pt dish to yield an excess of 0.5–5 ml. of the acid in the aliquot taken for precipitation. Wash into a small volumetric flask (25 ml. is satisfactory size), make to volume, and mix. Allow to stand at least 1 hour and filter, if necessary, through small paper filter. Withdraw a 10 ml. aliquot into a 50 ml. beaker (for precipitation aliquot should contain 24–18 mg. of K<sub>2</sub>O). Slowly add 5 ml. of the sodium cobaltinitrite solution\* from a pipet while stirring. Allow to stand for 2 hours at approximately 20° C. Protect the precipitating vessel from laboratory fumes by placing under a bell jar or similar device. Filter in a sintered-glass filtering crucible (Jena 1G4, 35 ml. capacity is convenient), the tare weight of which is known, using 0.01 *N* HNO<sub>3</sub> (approximately 20° C.) in a small wash bottle to make the transfer. (Volume of liquid used should be relatively small.) Wash ten times with 2 ml. portions of the dilute HNO<sub>3</sub>, and five times with 2 ml. portions of alcohol, releasing the vacuum each time before adding the washing fluid. Aspirate until quite dry. Dry 1 hour at 100°; cool in a desiccator and weigh.

If the K content of ash is low, obtain requisite quantity of K<sub>2</sub>O for precipitation by adding 2 ml. of the HNO<sub>3</sub> to the ash, diluting to 25 ml., and allowing to stand. After filtering, withdraw a 20 ml. aliquot into the 50 ml. beaker. Evaporate the solution to 10 ml. volume, previously marked on the beaker. After cooling to approximately 20°, complete the precipitation in the usual way. The composition of the precipitate is expressed as K<sub>2</sub>NaCo(NO<sub>2</sub>)<sub>6</sub>·H<sub>2</sub>O and mg. of precipitate  $\times 0.20738 \times 100$ /grams of sample in aliquot = mg. of K<sub>2</sub>O/100 grams of sample.

The potassium cobaltinitrite is conveniently removed from the crucible by dissolving in hot 5% by volume H<sub>2</sub>SO<sub>4</sub> in water. The crucible is then washed with distilled water, followed by alcohol, and dried.

## EXPERIMENTAL RESULTS OBTAINED

Known KCl solution 100.0 mg. K<sub>2</sub>O/100 ml.

(Aliquot of 10 mg. used)

mg. ml.

Analyst 1—100.0/100

Analyst 2—100.2/100

Analyst 3— 99.5/100

98.9/100

99.5/100

} Average 99.3 mg./100 ml.

The results obtained by the A.O.A.C. and cobaltinitrite methods by different analysts in widely separated sections of the country are given in Table 1.†

Wilcox also included in his paper a method for estimating the potassium content of the cobaltinitrite precipitate by titration with standard permanganate solution. Some analysts may prefer to determine the potash by the new method volumetrically instead of gravimetrically, or may

\* Since the details of the method were studied, it has been found that the contents of the beaker, as well as the cobaltinitrite solution should be cooled to 20° C. before the reagent is added.

† Abbreviations: N.Y. = New York Lab., temperature of washing and precipitation, 18–22° C.

C = Chicago Lab., temperature of washing and precipitate, 20° C.

S.F. = San Francisco Lab., temperature of washing and precipitate, 23–26° C

S.L. = St. Louis Lab.

w = Whole sample

s = Sample solution

XX = Results in different column by different analysts.

TABLE 1.—*Collaborative results*

NUMBER	COBALTNITRITE METHOD, XX	K <sub>2</sub> O FOUND IN MG./100 GRAMS OF SAMPLE A.O.A.C. CHLOROPLATINATE METHOD, XX	
FRUITS			
Peach			
N.Y. 1 s	183.0	180.6	182.1
Raspberry			
N.Y. 3 s	139.3	138.9	142.0
N.Y. 4 s	228.2	228.9	228.6
C. 1 s	191.8	188.1	
	(201.6)		
S.F. 1 s	226.0	224.2	224.5
	224.3		
Strawberry			
N.Y. 1 s	221.2	221.4	222.7
N.Y. 2 w	238.4	231.5	
N.Y. 3 s	213.8		214.4
			(199.7)
N.Y. 3 w	221.6	214.3	
N.Y. 4 s	230.1		226.3
N.Y. 5 s	181.5	177.3	
N.Y. 6 s	225.3	227.9	
N.Y. 6 w	225.2		
S.L. 1 w	166	162	
S.L. 1 s		157	158
S.L. 2 s		159	158
S.L. 3 w	165	163	
S.L. 3 s		154	149
S.L. 4 w	222	219	
S.L. 4 s		202	202
Plums			
N.Y. 1 s	205.0		196.2
N.Y. 2 w	275.7	279.9	272.0—S
Apricots			
N.Y. 1 s	566.8	566.2 w	557.5
N.Y. 2 s	563.5		573.6
S.F. 1 s	462.0	473	476.4 w
		468.0	
		468.7	
		475.6(1)	
		471.5(2)	
Youngberries			
S.F. 1 s	221.9	217.0	217.4
	216.3		
Pineapple Juice			
S.F. 1	217.6	220.7	221.7
	214.8		
Citrus Pectin			
S.F. 1	688.5	690.6	689.8
	661.5	694.6	

TABLE 1.—Continued

NUMBER	COBALTNITRITE METHOD, XX	K <sub>2</sub> O FOUND IN MG./100 GRAMS OF SAMPLE A.O.A.C. CHLOROPLATINATE METHOD, XX	
PRESERVES			
Peach			
N.Y. 2†w	114.9	116.4	120.4
N.Y. 2 s	116.1	115.4	114.5
	114.2		
N.Y. 3 w	97.7	95.5	98.5
Raspberry			
N.Y. 1‡s	43.8	43.9	
N.Y. 2‡s	39.4	39.3	
N.Y. 5 s	88.2	85.3	
N.Y. 6 s	112.9§	116.6	
Strawberry			
N.Y. 2 s	120.2	118.7	
N.Y. 3 s	122.8		120.5
N.Y. 3 w	122.3	121.8	
N.Y. 5 s	94.3	97.3	
N.Y. 7 s	94.0		92.9
N.Y. 8 s	109.7		111.2
N.Y. 9 s	118.0	121.3	
N.Y. 10 s	111.5		115.7
C. 1 s	95.6		95.5 (85.1)**
S.L. 5 w	120	118	
S.L. 5 s		117	116
S.L. 6 w	112	111	
S.L. 6 s		105	107.
S.L. 7 w	122	120	
S.L. 7 s		113	120
S.L. 8 s		109	112
S.L. 8 w	119	118	
Plum			
N.Y. 1 w	95.3	97.2	
N.Y. 2 w	140.1	140.8	
N.Y. 2 s	140.8		142.9
N.Y. 3 s	148.0		148.0
Cherry			
N.Y. 1 s	112.0	113.4	111.6
Apricot			
N.Y. 1 w	310.9		301.5
N.Y. 1 s	300.0		301.2
	300.7		
N.Y. 2 s	284.7		280.6
N.Y. 3 w	307.3		296.3

† Preserves are made from fruit of the same number.

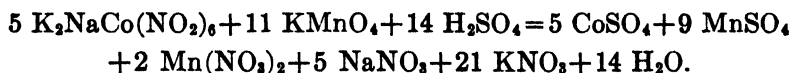
‡ Substandard.

§ Temperature of precipitation and washing fluid about 29° C.

(1) 28.54 mg. K<sub>2</sub>O precipitated, 10 ml. reagent used.(2) 28.29 mg. K<sub>2</sub>O precipitated, 5 ml. reagent used.

\*\* This value is low because of mechanical loss during removal of ammonium salts.

wish to use the titration as a check on the purity and composition of the weighted precipitate. Therefore a number of titrations of the precipitates obtained in the gravimetric procedure were made. The oxidation of the nitrite proceeds in accordance with the equation:



It will be noted that the cobaltic atom itself, being a powerful oxidizing agent, oxidizes an equivalent amount of nitrite.

While experimenting with the volumetric procedure and during routine gravimetric work, the writer observed that some of the potassium precipitates required large quantities of the dilute nitric acid for transferring to the crucible. In these cases, slightly low results were obtained. It was found to be advisable to use a saturated solution of pure potassium cobaltinitrite in dilute nitric acid for transferring and washing the precipitate.

The volumetric method as applied to fruit products is as follows:

#### REAGENTS

(In addition to those used in the gravimetric procedure, excepting 95% alcohol.)

(1) *Potassium cobaltinitrite solution*.—Saturated in 0.01 N  $\text{HNO}_3$ . Prepare the cobaltinitrite from a pure salt by precipitation as directed in the gravimetric method. Saturate the dilute acid solution by shaking vigorously 100 or 200 ml. with a few mg. of this precipitate for about an hour in a shaking machine. Filter through a Jena 1G4 crucible and prepare a fresh solution before each set of determinations. Transfer the precipitate obtained in the routine estimation with this solution (approximately 20° C.). Wash nine times with 2 ml. portions and then once with 1 ml. of the dilute  $\text{HNO}_3$  acid solution.

(2) *Potassium permanganate*.—0.05 N. Standardize against sodium oxalate.

(3) *Sodium oxalate solution*.—0.05 N.

(4) *Sulfuric acid*.—50% by volume.

(5) *Sodium hydroxide solution*.—Approximately 0.5 N.

#### PROCEDURE

Follow the gravimetric technic through the precipitation and washing. Omit the alcohol treatment unless the precipitate is to be dried and weighed. In either case wash the nitrite from the crucible into a 250 ml. beaker, place the crucible therein, and make to about 100 ml. with water. Add 20 ml. of 0.5 N NaOH and boil for 3 minutes.

Withdraw into a 400 ml. beaker a slight excess of the standard permanganate. Pour the hot (still boiling) alkaline solution into the oxidizing mixture and rinse with a little water. Immediately add from a pipet, with stirring, 10 ml. of the  $\text{H}_2\text{SO}_4$ , keeping the tip of the pipet below the surface of the liquid. Warm to 90° C., then add an excess of sodium oxalate. Heat to boiling and complete the titration with the permanganate solution. (The method employed in acidification, together with heating to 90°, appears in a large measure to eliminate the slightly low results that were sometimes obtained without these refinements of the simple titration. This was noted especially on some of the larger precipitates and may possibly have been due to incomplete oxidation or loss of nitrous acid during acidification.)

Run a blank in a similar way, using 1 or 2 ml. of permanganate to receive the boiling alkaline solution. ml. of 0.05 *N*  $\text{KMnO}_4$  (less blank, less oxalate)  $\times 0.42814$  = mg. of  $\text{K}_2\text{O}$ .

The results obtained with this titration on precipitates of potassium cobaltinitrite are shown in Table 2.

TABLE 2.—*Results obtained on precipitates of potassium cobaltinitrite*

DESCRIPTION		WEIGHT OF K <sub>2</sub> O IN PRECIPITATE (MG.)	
NUMBER	KNOWN K <sub>2</sub> SO <sub>4</sub> SOL. ALIQUOT OF 9 MG.	BY WEIGHING 8.98	BY TITRATION OF THE SAME PRECIPITATE 9.00
<i>Fruits</i>			
(1) w	Blackberry	18.43	18.30
(1) w	Black Raspberry	9.83	9.81
(1) s	Cherry	8.03	8.04
(1) w	Peach	14.41	* 14.90
		15.20	
		15.10	
(1) w	Raspberry	15.14	15.10
<i>Preserves</i>			
(1) w	Blackberry	4.06	4.09
(1) w	Black Raspberry	9.83	9.81
(2) w	Cherry	11.28	11.29
(3) s	Cherry	10.22	10.19
(1) w	Seedless Black Raspberry	10.41	10.40
(1) s	Seedless Black Raspberry	9.99	9.93
(1) w	Apricot	14.70	14.62
(1) w	Strawberry	8.65	8.64
(1) w	Raspberry	8.07	8.08
(1) s	Raspberry	5.39	5.39
<i>Pectins</i>			
(1) ———	—	8.38	8.40
(2) ———	—	11.53	11.52

\* Chloroplatinate method results calculated to aliquot taken for titration.

The range of the Wilcox method from 2.4 to 18 mg. of potassium oxide is convenient for the analysis of preserves and nearly all fruits. An aliquot equivalent to 6–10 grams of material requires a factor from 10 to 17 to convert the milligrams of  $\text{K}_2\text{O}$  found to milligrams per 100 grams of fruit or preserves. However, occasionally, as in the case of apricots, the potassium content may be two or more times that of the other fruits. The aliquot necessarily taken for analysis may therefore be much smaller than 6 grams.

In order to increase the quantity of potassium precipitated in such cases, experiments were conducted with larger volumes of solution and larger quantities of cobaltinitrite in the determination. Stoichiometric results were obtained when a 30 ml. volume was used for precipitation

and 15 ml. of reagent containing 6 grams of sodium cobaltinitrite (proportionally greater amounts of cobaltinitrite were used here to minimize the solubility of the potassium precipitate). The addition of the precipitating reagent should be drop by drop from a buret or pipet to minimize coprecipitation. The saturated potassium cobaltinitrite solution for washing should be used in fourteen 3 ml. portions. Otherwise, the procedure is just as previously described. The range of concentration tested extends from 18 to 45 mg. of  $K_2O$ . Much above this amount, the potassium recovered by weighing tends to become low.

Recoveries on known solutions were as follows:

Concentration of acid.....	3 ml. of 1 N $HNO_3$
Temperature.....	$20^\circ \pm 2^\circ$
Time of precipitation.....	2-3 hours

<i>Mg. <math>K_2O</math> Added as KCl</i>	<i>Mg. Recovered</i>
18.00	18.00
20.00	20.08
25.00	25.10
30.00	29.95
35.00	35.03
40.00	39.88
45.00	44.92

<i>Results on Fruit Ashes</i>	<i>Cobaltinitrite Method</i>	<i>Chloroplatinate Method</i>
1. Solution obtained from mixed fruit ashes (apricot, raspberry, strawberry, cherry), aliquots equal to 20 grams of fruit	152.5 153.0	150.6 153.0
2. Solution from cherry ash plus large amounts of KCl cobaltinitrite aliquot = 6 gram chloroplatinate aliquot = 7.5 gram	560.0 560.3	554.2 553.6
Theoretical amount of $K_2O$ present = 200 (average of 2 cobaltinitrite and 2 chloroplatinate determinations) plus 360 mg. added as KCl	560	560
Average % recovery of total $K_2O$ present	100.0	98.9

#### LIMITATIONS OF THE METHOD AS INDICATED BY EXPERIMENTS ON FRUIT PRODUCTS

(1) *Temperature*.—Slightly high results will be obtained at  $15^\circ$  (on the order of 1 per cent). Slightly low results will be obtained at higher temperatures, i.e., at  $25^\circ$  results are about 1 per cent low. Therefore, the temperature of precipitation and washing is set at  $20^\circ \pm 2^\circ$ .

(2) *Time of Precipitation*.—Within 2-4 hours practically the same results are obtained. On standing overnight, results may be slightly high.

(3) *Type of Crucible*.—The 1G4 sintered-glass crucible was found to be

most satisfactory for this estimation. Smaller sizes of disk filter quite slowly, and those of greater porosity will not retain the precipitate, which at times may be quite fine, on the filter. In fact, analysts are warned that the porosity of this style crucible, in a given grade, may vary considerably either from normal variation or changes with continual use. Any crucibles that filter unusually fast should not be used for this estimation. Gooches packed with asbestos have been used. The precipitate on the asbestos mat, however, may require more washing than does the one retained on the sintered glass.

(4) *Time of Drying the Precipitate.*—One hour is sufficient, but the precipitate may be dried for several hours without apparent change.

### CONCLUSION

The Wilcox cobaltinitrite volumetric and gravimetric methods for the determination of potassium, herein applied to the analysis of fruit products, requires less working time and analytical manipulation than does the A.O.A.C. chloroplatinate procedure for this metal. Check analyses between the A.O.A.C. estimation and the cobaltinitrite precipitation by laboratories in widely different areas indicate good agreement.

The writer wishes to express appreciation for the advice given by J. Fitelson and G. Kirsten of the New York Station.

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### VOLATILE OIL IN CASSIA BARK

By J. F. CLEVINGER (U. S. Food and Drug Administration,  
New York, N. Y.)

During the past six years analyses have been made of representative samples of many shipments of cassia barks entering the port of New York. The yield of volatile oil and the physical and chemical characteristics of these oils were determined.

Based upon the appearance of the bark, the characteristics of their volatile oil content, and their geographic origin these barks may be grouped into seven rather definite classes. Saigon, Honan, China, Kwongsai, Korintji, and Batavia are usually referred to as cassia and Ceylon is usually referred to as cinnamon. In addition, limited quantities of cassia buds are imported. These barks come into the market whole and sometimes variously bundled. In the same shipment are frequently found bales of broken barks varying from  $\frac{1}{3}$  the usual length (approximately 1 foot) to short pieces.

The volatile oil obtained from these barks and buds must not be confused with the volatile oil official in U.S.P. XI as Oil of Cinnamon, which is obtained from the leaves and stems of *Cinnamomum cassia*. Volatile oils obtained from the latter are liable to vary considerably in their physical



and chemical characteristics. Data on oils from this source were not obtainable since the leaves and stems were not available.

Saigon cassia (Figure 1) comes in quills varying in thickness from 1 to 5 mm. and always unrossed. The quills are usually tied in bundles and are medium dark brown in color. This is a high-grade bark.

China cassia (Figure 2) is similar in appearance to Saigon cassia, but the quills are of a more uniform thickness, approximately 2 mm. It is seldom put up in bundles. In addition, a large percentage of broken cassia is imported and is graded into common broken, select broken, and extra broken. This classification is based upon appearance, and means little in relation to spice value.

Honan cassia (Figure 3) is grown in the Honan district in China. The bark is rossed, light brown in color, fairly uniform in thickness, usually strung on strings, of a high grade, and has a pleasant taste. This bark is imported in relatively small quantities.

YIELD v/w*	SP. GR. 25°/25° C.	OP. ROT. 25° C.	REF. IND. 20° C.	ALDEHYDE	NON-ALDEHYDE	
					ACID. NO.	ESTER NO.
Saigon Cassia						
1.5	1.051	0.0	1.610	96	2.2	230
3.0	1.056	0.0	1.606	97	6.5	163
2.8	1.054	0.0	1.607	96	4.2	223
4.0	1.056	0.0	1.609	95	5.3	183
3.0	1.052	0.0	1.605	94	5.1	163
2.9	1.057	0.0	1.609	96	4.7	193
3.0	1.056	0.0	1.612	98	6.2	100
3.0	1.056	0.0	1.607	90	4.4	144
3.0	1.051	-0.6	1.610	97	8.0	173
2.5	1.047	0.0	1.611	97	10.1	222
Honan Cassia						
2.0	1.043	-0.6	1.608	96	11.3	28.5
2.6	1.043	0.0	1.606	97	11.8	34.0
3.1	1.043	0.0	1.605	98	11.2	33.6
3.0	1.043	0.0	1.606	96	6.7	47.3
3.0	1.051	0.0	1.614	98	11.7	34.1
China Cassia						
1.5	1.049	-0.6	1.609	—	6.7	130
0.9	1.049	0.0	1.611	95	12.6	67
1.0	1.047	-0.6	1.610	96	13.4	67
0.9	1.047	-0.7	1.606	97	19.5	41
1.5	1.052	-0.6	1.610	97	8.2	108
0.8	1.043	0.0	1.610	96	14.9	50
0.8	1.047	0.0	1.611	97	12.2	39
0.75	1.043	-0.6	1.608	96	3.9	40
1.0	1.047	0.0	1.611	97	12.0	69
1.5	1.051	-1.0	1.608	97	13.0	92

YIELD v/w*	SP. GR. 25°/25° C.	OP. ROT. 25° C.	REF. IND. 20° C.	ALDEHYDE	NON-ALDEHYDE	
					ACID NO.	ESTER NO.
<i>Cassia Buds</i>						
1.6	1.038	0.0	1.596	87	3.7	70
1.2	1.032	0.0	1.595	87	7.8	27
0.8	1.042	-0.6	1.597	96	5.2	82
1.1	1.042	-0.7	1.601	95	5.8	41
0.8	1.045	-1.0	1.610	97	7.7	60
1.0	1.038	-1.0	1.597	89	5.5	28
1.0	1.035	-0.7	1.606	93	4.6	43
1.3	1.037	-0.4	1.595	90	4.8	36
1.2	1.047	-0.6	1.603	96	5.6	41
1.0	1.043	-1.0	1.604	94	2.7	79
<i>Kwongsai Cassia</i>						
2.5	0.987	-3.5	1.559	60	—	—
1.0	1.004	-0.7	1.573	68	1.4	12.8
0.8	1.043	-1.3	1.604	94	5.8	26.1
1.0	0.983	0.0	1.553	60	0.9	12.8
1.1	1.026	-1.0	1.598	89	4.2	34.8
1.0	1.051	-0.6	1.614	98	8.4	56.1
1.49	1.038	-0.3	1.603	92	4.6	44.2
1.3	1.045	-1.7	1.607	90	7.3	24.4
1.0	1.032	-1.3	1.596	86	2.3	55.3
<i>Korintji Cassia</i>						
2.5	1.030	-3.4	1.598	90	5.2	58.9
2.1	1.030	-3.4	1.598	88	6.5	49.8
1.0	1.025	-4.8	1.587	90	7.6	79.5
2.0	1.038	-2.7	1.600	92	8.3	93.1
1.8	1.037	-3.8	1.590	90	10.8	99.7
2.0	1.030	-2.7	1.592	90	8.7	70.1
<i>Batavia Cassia</i>						
1.7	1.027	-4.0	1.588	88	6.0	131
1.9	1.027	-3.0	1.593	90	5.0	84
0.9	1.024	-4.1	1.582	84	4.8	99
0.6	1.030	-4.8	1.586	80	3.9	81.0
1.8	1.034	-2.8	1.595	83	8.6	70.0
0.95	1.023	-3.2	1.587	78	4.6	63
1.5	1.027	-3.5	1.595	80	5.6	102
1.3	1.034	-3.5	1.596	86	5.4	95
1.7	1.027	-3.1	1.590	82	10.0	121
1.1	1.034	-1.4	1.596	85	7.6	89
<i>Ceylon Cinnamon</i>						
1.3	1.030	-0.7	1.584	68	3.98	136
1.3	1.022	0.0	1.582	66	10.0	145
1.2	1.027	-0.6	1.586	68	5.6	127
1.2	1.027	-0.6	1.585	70	6.4	127
1.3	1.021	-0.6	1.579	72	5.8	134
1.2	1.017	0.0	1.589	76	—	—

\* Ml. per 100 grams of cinnamon.

Kwongsai cassia (Figure 4) is grown in the Kwongsai district of China, is usually very rough in appearance, and is approximately 5 mm. in thickness. Considerable and increasing quantities of this bark are being imported. It is usually considered to be an inferior cassia.

The Korintji (Figure 5) and Batavia (Figure 6) cassias, grown in the East Indies, differ from the above varieties in that the barks contain large quantities of mucilage and are lighter brown in color. Korintji bark comes in thick (approximately 3 mm.) fairly uniform, unrossed quills. The Batavia bark comes in thin (approximately 1 mm.) rossed quills. A large proportion of broken Batavia cassia is imported. It is graded into classes based primarily on appearance, which means little in regard to spice value.

Ceylon cinnamon (Figure 7), grown in Ceylon, is usually referred to as cinnamon and is characterized by the many parallel-layered groupings of this very thin bark. This bark, as imported, is of a fairly uniform quality. It is considered valuable because of its desirable bouquet. A large proportion of this bark is said to be re-exported.

The results reported in the table were obtained by the methods outlined in *Methods of Analysis, A.O.A.C.*, 1935, 447-449.

#### CONCLUSIONS

(1) The physical and chemical characteristics for each class of bark here reported are fairly uniform, except for the oils of the Kwongsai cassia. This bark shows considerable variation for all constants.

(2) The oils from the cassias grown in the East Indies differ from those grown in China in having a lower specific gravity, index of refraction, and aldehyde content, and also in having a distinct negative rotation.

(3) Ceylon cinnamon is characterized by a reasonably uniform yield of volatile oil having a low aldehyde content.

(4) The characteristics of the volatile oil should serve as an aid in the identification of unknown samples of cassia.

(5) The cassia barks grown in the East Indies show a large mucilage content.

(6) None of the oils here reported may be considered as the official Oil of Cinnamon in U.S.P. XI.

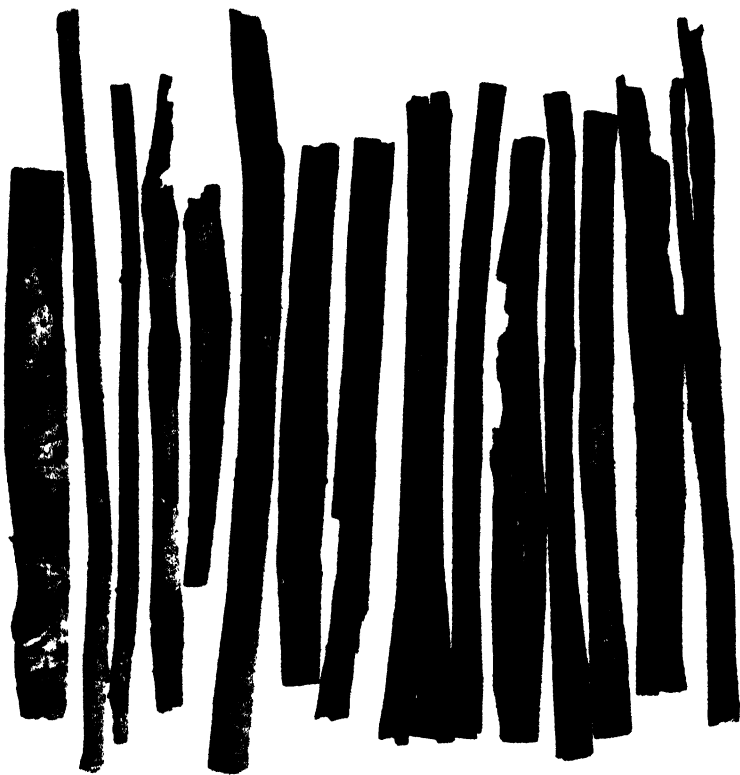


FIG 1 SAIGON CASSIA

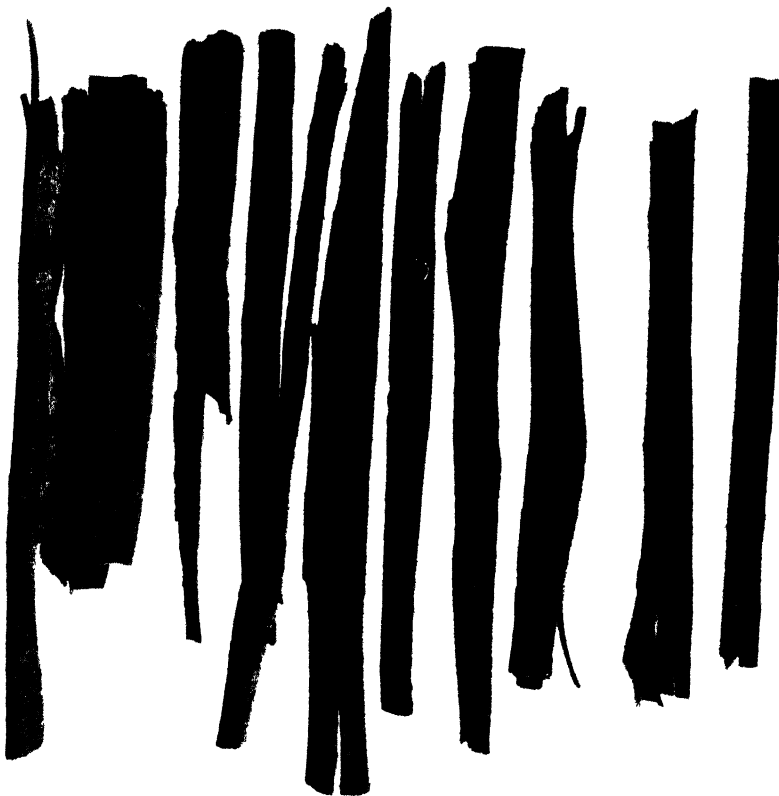


FIG. 2. CHINA CASSIA



FIG. 3.- HONAN CASSIA



FIG. 4 KWONGSAI CASSIA



FIG. 5 KORINTJI CASSIA

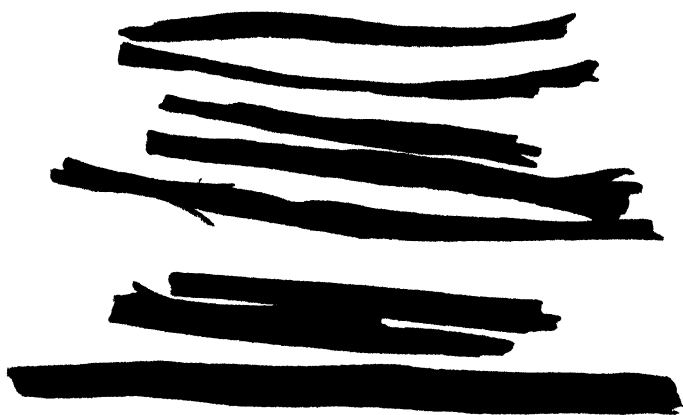


FIG. 6. - BATAVIA CASSIA.

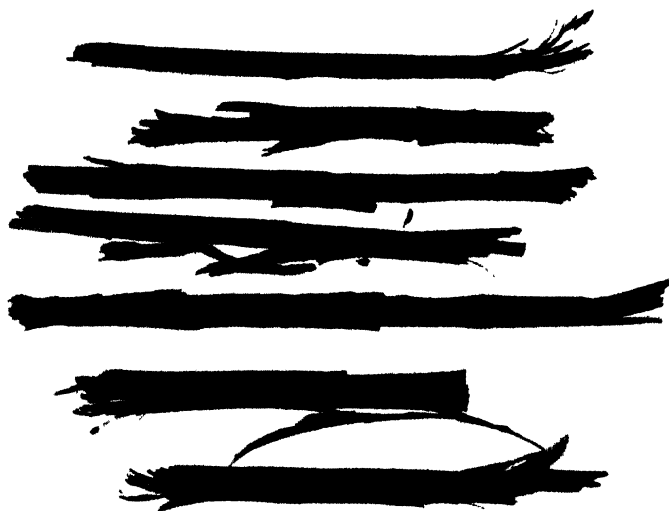


FIG. 7. CEYLON CINNAMON.

## THE ASSAY OF PEPPERMINT, ROSEMARY, AND SANDALWOOD OILS

By DONALD C. GROVE (U. S. Food and Drug Administration,  
Washington, D. C.)

The U.S.P. XI (1) assay of the above oils is based on the classical method of acetylation by means of acetic anhydride and anhydrous sodium acetate. The acetylated oil is washed free of excess acetic anhydride by means of water and sodium carbonate solution and dried with anhydrous sodium sulfate, and a weighed portion of the dry acetylated oil is quantitatively saponified with 0.5 *N* alcoholic potassium hydroxide.

There are several criticisms to be made of this method. First, there is the possibility that other constituents of the oil beside the alcohols are capable of acetylation. Thus, Delaby and Breugnot (2) found that the French Codex method, which is essentially the same as the U.S.P. XI method, was capable of partially acetylating the hydrocarbon santalene, a normal constituent of sandalwood oil. Aldehydes are also capable of acetylation by this method.

Second, the percentage of alcohols present is determined by saponifying a weighed sample of acetylated oil that has been washed with a rather strong solution of acetic acid, formed by the decomposition of the excess acetic anhydride in the added water, and with a solution of sodium carbonate. Thus, the accuracy of the method is based upon the assumption that the concentration of the acetates of the alcohols in the acetylated, washed mixture is a measure of the concentration of the alcohols in the original oil. For this assumption to be true, it is essential that the distribution coefficient of alcohols (as their acetates) between the aqueous wash solutions and the oily residue be the same as the average of the coefficients for all of the other constituents originally present in the oil. That this is probably the case with most oils is not questioned, but this weakness in the method precludes the detection of an oil having the specified physical constants, but containing an acid or alkali soluble constituent, either naturally present or added as an adulterant.

Third, Baldinger (3) believed that in the saponification of the acetylated oil with alcoholic potassium hydroxide, resinification or polymerization of certain constituents, evidenced by a darkening of the reaction mixture, was induced, and that in the reaction some base was used up, thereby leading to erroneous results. He based this argument on the fact that saponification longer than 60 minutes with alcoholic potassium hydroxide gave a higher result. These slightly higher results may, however, be due to the presence of other menthol isomers. Hall, Holcomb, and Griffin (4) have shown that saponification of *d*-neomenthyl acetate with 0.5 *N* alcoholic potash for one hour gave only 69 per cent *d*-neomenthol, and that 3.5 hours was required for complete saponification. They recom-



mended the use of a diethylene glycol solution of potassium hydroxide, whereby saponification was complete in from 5 to 30 minutes.

A final criticism of the U.S.P. XI method is that it is cumbersome and very time consuming.

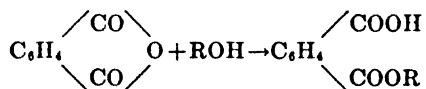
A review of the literature for more convenient and accurate methods was made. It was found that Verley and Bolsing (5), following the proposal of Einhorn and Hollandt (6) of acetylation in the presence of pyridine, used a method for the determination of alcohols and phenols in essential oils based on acetylation with acetic anhydride and pyridine. They weighed 1-2 grams of the alcohol into a flask of about 200 ml. capacity and added 25 ml. of a mixture consisting of 120 grams of acetic anhydride and 880 grams of pyridine, warmed on the water bath without a condenser for 15 minutes; then, after cooling, they added 25 ml. of water and titrated the acetic acid formed with normal sodium hydroxide, using phenolphthalein as indicator. The pyridine aided in the acetylation by forming pyridine acetate with the liberated acetic acid and prevented the loss of acetic acid or acetic anhydride when the sample was heated in an open flask on the steam bath for as long as 3 hours. The pyridine also produced an immediate decomposition of the excess acetic anhydride when the water was added to it, so that the solution could be titrated at once. Their method applied to santalol gave results of 94.8 per cent and 95.4 per cent. With sandalwood oil, they obtained 80.75 per cent santalol. In the case of menthol, they found that a 500 per cent excess of acetic anhydride was necessary for complete acetylation.

Van Urk (7), in applying the Verley-Bolsing method (5) for the quantitative esterification of the alcohols in oils of peppermint, clove, and sandalwood, found that it did not give quantitative results. The addition of 50 ml. of reagent gave higher results, although still too low. He claimed that the method was only of use for the approximate analysis of clove and sandalwood oils, but not peppermint oils. He found 34.5 per cent and 40.6 per cent of menthol in oil of peppermint and 87.76 per cent and 91.1 per cent santalol in oil of sandalwood by this method. By the Pharmacopoeial method he obtained 47.1 per cent free menthol and 92.3 per cent santalol.

Later, Verley (8), in order to obtain more complete acetylation of certain primary and secondary alcohols, modified the procedure by using a reagent composed of two parts of pyridine, to which was added one part of acetic anhydride containing 5 per cent of acetyl chloride. He used 10 ml. of this reagent for 2-3 grams of alcohol and heated it on the bath with a condenser for varying periods of time, depending upon the nature of the alcohol. Figures were given for a technical santalol on which he obtained 82.61 per cent after heating for one hour and up to 95.81 per cent after heating 4 hours. On menthol he obtained 94.41 per cent after 2 hours and 100.05 per cent after 4 hours' heating.

Delaby and Sabetay (9) recommended the use of the Verley-Bolsing method (5) for the specific determination of primary and secondary alcohols. The reagent they used consisted of 2 parts of pyridine and 1 part of acetic anhydride by weight. They heated on the water bath under a condenser for one half to one hour, added 50 ml. of water through the top of the condenser, and heated for an additional 15 minutes on the bath to decompose the excess acetic anhydride before titration. This latter step was necessary they contended because the addition of water in the cold did not decompose the acetic anhydride immediately, as stated by Verley and Bolsing. Delaby and Sabetay also claimed that under the experimental conditions which they used, the primary alcohols, primary amines (methyl anthranilate, for example), and phenols acetylated quantitatively in one-half to one hour, the secondary alcohols nearly quantitatively in about 1 hour, and finally that the tertiary alcohols and aldehydes were scarcely touched. They also believed that pyridine acted as a negative catalyst, which paralyzed the acetylation of tertiary alcohols by acetic anhydride. They obtained 97.6 per cent and 95.6 per cent on menthol after heating one-half hour and 96.4 per cent after three-quarters of an hour. For borneol they obtained 95.9 per cent after heating for one hour. Figures were given for some tertiary alcohols and aldehydes showing very little or no acetylation of these compounds. These authors also stated that the replacement of the acetic anhydride by benzoic anhydride did not give satisfactory results and the addition of acetyl chloride to the acetic anhydride-pyridine mixture, as suggested by Verley (8), did not offer any advantage.

Stephen (10) observed that certain primary alcohols reacted quantitatively with phthalic anhydride at 80° C. to give acid phthalates according to the following equation:



Schimmel and Co. (11) proposed a procedure for the determination of primary alcohols, particularly geraniol in oil of citronella, by heating with phthalic anhydride in the presence of benzene. Radcliffe and Chadderton (12) modified the Verley-Bolsing method (5) by using a mixture of phthalic anhydride and pyridine. They recommended using 25 ml. of a reagent consisting of 50 grams of phthalic anhydride in 250 ml. of pure pyridine. Two to three grams of sample was taken, and the mixture was allowed to stand at room temperature for 18 hours, then water was added, and the excess acid was titrated. They obtained satisfactory results in the case of pure geraniol, pure citronellol, and mixtures of citronellol and citronellal.

Glichitch and Naves (13) have investigated both the phthalization method recommended by Schimmel & Co. (11) and the pyridine-phthali-

zation method of Radcliffe and Chadderton (12). They found that the Schimmel & Co. method was not particularly satisfactory for primary alcohols and was very irregular for secondary alcohols; thus, menthol gave 39.6 per cent and 35.2 per cent and borneol gave 46.2 per cent and 52.7 per cent. With the Radcliffe-Chadderton method they obtained very satisfactory results for a large number of primary alcohols, the santalols giving the lowest result in the list (97.6 per cent). For menthol, however, only 88.5 per cent was obtained. They also listed a large number of tertiary alcohols, aldehydes, oxides, lactones, phenols, and esters that did not react with the reagent. Among the oils examined by them was a sample of sandalwood oil (Mysore) in which they found 86.4 per cent santalol.

Delaby and Breugnot (2), in a paper dealing with the determination of alcohols in sandalwood oil, compared results obtained by the methods of the French Codex, Verley and Bolsing, Delaby and Breugnot (a modification of the Verley-Bolsing method) and Radcliffe and Chadderton. The results from the last three methods showed good agreement among themselves, but they are at least 10 per cent lower than the results from the Codex method. They were able to show that the Codex method included, in addition to primary alcohols, any santalol combined as esters and a portion of the hydrocarbons such as santalene. They recommended their method of acetylation with pyridine and acetic anhydride and stated that the official oil of sandalwood should give 79-80 per cent santalol by this method, a result which they claimed was closer to the truth than the 90 per cent given by the Codex method.

Sabetay and Naves (14) investigated the benzene-phthalization method of Schimmel & Co. (11) and modified it by adding pyridine to the mixture after phthalization to break down the excess phthalic anhydride before titration. However, they did not obtain satisfactory results by this method. The same authors then decided to modify the Radcliffe-Chadderton method (12) by using hot pyridinic phthalization. They prepared the reagent by dissolving 50 grams of phthalic anhydride in 250 ml. of pure pyridine, dried by distillation over barium oxide, and filtered to eliminate the slight excess of phthalic anhydride that did not dissolve. One-half to one gram of the alcohol or an amount of essential oil containing this quantity was placed with 10 ml. of the reagent (2-4 times the theoretical quantity of phthalic anhydride) into an acetylation flask connected to a condenser by means of a ground-glass joint. The flask was dipped into boiling water for 1 hour. Then 50 ml. of water was added, and the mixture was allowed to remain 10 minutes longer in the boiling water. After being cooled, the mixture was transferred to a 250 ml. beaker, the condenser and flask were washed, and the washings were added to the beaker. The excess acid was titrated with 0.5 *N* sodium hydroxide, phenolphthalein being used as indicator. They presented a table of analyses by this method of

various primary and secondary alcohols and oils. Using pure alcohols, they recovered 98.0 per cent of menthol and 78.5 per cent of borneol, and found in oil of sandalwood (Mysore) 76.5 per cent of santalol.

Sabetay (15) more recently has recommended a change in the above method of hot pyridinic phthalization. After the reaction has proceeded for one hour, 50 ml. of water is added, and the mixture is heated only 1 minute in the water bath, cooled, and titrated. He found that heating 10 minutes after adding the water is liable to cause hydrolysis of some of the acid phthalate ester. He also recommended an excess of phthalic anhydride corresponding to four times the theoretical.

Smith and Bryant (16) recommended the use of acetyl chloride and pyridine for the determination of primary and secondary alcohols because of its faster reaction. They used 10 ml. of 1.5 *M* acetyl chloride in toluene, 2 ml. of pyridine, and a weight of sample equivalent to not more than two-thirds of the acetyl chloride. The mixture was heated at 60° C. for 20 minutes and cooled; water was added, and the excess acid was titrated with standard alkali. They obtained 98.4 per cent for borneol.

Tertiary alcohols are not completely esterified by acetic anhydride and anhydrous sodium acetate because of partial dehydration and subsequent hydrocarbon formation. Thus Glichitch (17) claimed that only about 74 per cent of linalol is obtained by this method. He recommended the use of acetoformic anhydride reacting in the cold for 72–96 hours for the quantitative determination of tertiary alcohols.

Boulez (18), in the determination of tertiary alcohols, advocated dilution of the sample with oil of turpentine or xylene (an indifferent medium) prior to acetylation with acetic anhydride and sodium acetate. He claimed quantitative results with this method.

#### EXPERIMENTAL

From the methods reviewed it seemed that the pyridine-acetyl chloride, the pyridine-acetic anhydride, and the pyridine-phthalic anhydride methods offered the most promise. Accordingly, these methods were investigated.

*Pyridine-acetyl chloride method.*—The reagent of Smith and Bryant (16) was modified by the use of carbon tetrachloride in place of toluene and a weaker solution of acetyl chloride. The mixture was allowed to stand at room temperature instead of being heated to 60° C. The procedure used was as follows: 25 ml. of 2 per cent v/v solution of acetyl chloride in carbon tetrachloride was pipetted into a 250 ml. glass-stoppered volumetric flask and cooled 5 minutes in ice water, and 15 ml. of a 5 per cent v/v solution of pyridine in carbon tetrachloride was added. The mixture was shaken thoroughly, then 0.5–0.75 gram of 1-menthol was added, followed by 10 ml. of carbon tetrachloride to wash down any sample adhering to the neck of the flask. The flask was stoppered and allowed to stand at

room temperature (about 25° C.) for 1 hour with occasional shaking. Then the flask was cooled several minutes in ice water, 50 ml. of water was added, and the contents were shaken thoroughly and titrated with 0.5 N NaOH, phenolphthalein being used as indicator. A blank was run simultaneously.

Using this method, the writer obtained recovery of 99.0 per cent on 1-menthol. The method was next applied to an oil of peppermint that analyzed 57.4 per cent of total menthol by the U.S.P. XI method. The amount of free menthol, correcting for the esters, was 52.7 per cent. By the pyridine-acetyl chloride method above, the oil gave 41.6 per cent menthol. When allowed to stand for two hours, 43.1 per cent was obtained and standing overnight (17 hours) gave 46.5 per cent menthol. Another sample was heated at 60° C. for 30 minutes and gave 38.2 per cent menthol.

A stronger solution, approximately 10 per cent w/v of acetyl chloride in carbon tetrachloride, was prepared. Ten ml. of this reagent was pipetted into the flask and to it was added 2 ml. of pure pyridine, the sample, and finally 10 ml. of carbon tetrachloride. This gave a smaller volume of solution and a greater concentration of acetyl chloride. Using this reagent, the writer obtained 47.0 per cent menthol on the oil after it had stood 1 hour at room temperature. When allowed to stand overnight (18 hours) 51.1 per cent menthol was indicated. The substitution of toluene for the carbon tetrachloride gave results of the same order.

Further work with acetyl chloride was not attempted, because it was believed the method, in which some loss of acetyl chloride was almost unpreventable, was not capable of giving as great accuracy as other methods in which the esterifying agent and the pyridine could be added as one reagent.

*Pyridine-acetic anhydride, reagent A.*—This reagent was prepared by mixing 10 ml. of acetic anhydride with enough C.P. pyridine to make 100 ml. As a large excess of acetic anhydride is always present, traces of water will not interfere with the determination. However, if the pyridine is suspected of containing more than a trace of water, it should be dried over barium oxide for several days and then distilled. No significant difference was observed in results with C.P. pyridine without drying, as compared to the same pyridine dried and distilled over barium oxide. Reagent A turns brown after 1 or 2 days' standing, and as the brown color may interfere in the determination of the end point of the titration freshly prepared reagent was used for the assays.

The procedure used with this reagent in the assay of alcohols and volatile oils was as follows: 0.5 gram of the alcohol or an amount of volatile oil equivalent to this quantity of alcohol (1.0 gram of peppermint and sandalwood oils and 4.0 grams of oil of rosemary) was accurately weighed into a 200 ml. round-bottomed flask with a ground joint; 10 ml. of reagent

A was accurately pipetted into the flask, which was then connected to a 400 mm., water-cooled reflux condenser, a little petrolatum being used on the joint. The flask and condenser were placed on a steam bath, the flask dipping about half way into the bath, and heated for 0.5–2.0 hours. At the end of this time, the flask was raised several inches from the bath and allowed to cool for 15 minutes; 50 ml. of water was added through the top of the condenser; the flask was disconnected; and the joint was washed with water. The mixture was then titrated with 0.5 *N* sodium hydroxide, phenolphthalein being used as indicator. A blank determination was run at the same time.

The effect of actually boiling the mixture with a flame was also studied. The same procedure given above was used except that instead of heating on the steam bath, the flask was placed on an asbestos board having a hole about 1.25 inches in diameter and just brought to boiling with a micro-burner. It was found that there was no loss of acetic anhydride or acetic acid after boiling for 2 hours under a reflux condenser. The contention of Verley and Bolsing (5) that the mixture may be titrated immediately after the addition of water was confirmed.

To study the effect of increasing the strength of the acetic anhydride, another reagent was prepared containing 14 ml. of acetic anhydride diluted with pyridine to make 100 ml. Results obtained with this reagent were of the same order as those found with reagent A, indicating that an increase in the concentration of acetic anhydride did not increase the degree of acetylation. The solution used by Delaby and Sabetay (9), containing 2 parts of pyridine and 1 part of acetic anhydride, due to the difficulty involved in measuring the small amount of the reagent required, did not permit the degree of accuracy obtainable with more dilute reagents.

*Pyridine-acetic anhydride-xylene, reagent B.*—This reagent was prepared by mixing 10 ml. of acetic anhydride and 20 ml. of pyridine and diluting with xylene to 100 ml. The purpose of the xylene was to raise the boiling point of the mixture and determine the effect of this factor on the acetylation. The boiling point obtained when the U.S.P. XI method was used was 146° C. The boiling point of mixtures of peppermint oil and these reagents was found to be 116° C. with reagent A and 127° C. with reagent B. In order to raise the boiling point of the pyridine-acetic anhydride mixture still higher, a reagent was prepared with 10 ml. of acetic anhydride, 20 ml. of pyridine, and enough *p*-cymene to make 100 ml. This, when mixed with oil, boiled at 138° C. However, as the results obtained with this reagent were the same as those obtained with reagent B, it was seen that nothing was to be gained by increasing the boiling point past that of reagent B and the use of the *p*-cymene reagent was discontinued. Reagent B keeps much better than reagent A. It will remain practically colorless for a week or two and then gradually turn yellow. The method

of assay with this reagent was exactly the same as that described under reagent A.

*Pyridine-phthalic anhydride, reagent C.*—This reagent was a 20 per cent solution of phthalic anhydride in pyridine. As the reagent turns yellow in 1 day, it should be freshly prepared before use. The method of assay used with this reagent is essentially that described by Sabetay (15). The same procedure was followed as under reagent A; 10 ml. of reagent was used for 0.5 gram of the alcohol or equivalent amount of oil except that after the mixture had been heated for the prescribed length of time on the bath 50 ml. of water was added through the top of the condenser, and the mixture was further heated for *one minute*, disconnected, cooled under the tap, and titrated immediately. When the mixture has been heated over a flame, the flame is turned out, 50 ml. of water is added, and the mixture is allowed to stand 1 minute, disconnected, cooled under the tap, etc.

The reagents A, B, and C were used to assay the following substances: *l*-menthol, *d*-neomenthol, borneol, oil of peppermint, oil of rosemary, and oil of sandalwood. The *l*-menthol and borneol were obtained from the Eastman Kodak Co. The *d*-neomenthol was obtained from the Swann Co.; it had a specific gravity at 25° C. of 0.897 and a specific rotation $[\alpha]_D^{25^\circ}$  of +19.6.

The peppermint oil was obtained from the A. M. Todd Co. and was labeled as "Crystal White Rose Mitcham Oil of Peppermint." The refractive index at 20° C. was 1.4600; the specific gravity at 25° C. was 0.903. It contained 6.3 per cent of esters calculated as menthyl acetate. Total menthol, free and as esters by the U.S.P. XI method, was found to be 55.2 per cent (av. 2 determinations). Free menthol, calculated as difference between total menthol and esters, was 50.2 per cent.

The oil of rosemary was obtained from Fritzsche Brothers, Inc. and was labeled "Oil of Rosemary Flowers U.S.P." The refractive index at 20° C. was 1.4695, and the specific gravity at 25° C. was 0.911. Analysis by U.S.P. XI method gave esters as bornyl acetate, 2.5 per cent; and total borneol free and as esters, 13.8 per cent (av. 2 determinations). Free borneol, calculated from the above figures, was 11.8 per cent.

The oil of sandalwood was obtained from Fritzsche Brothers, Inc., and was labeled "Oil of Santal, East India U.S.P." The refractive index at 20° C. was 1.5058 and the specific gravity at 25° C. was 0.972. The U.S.P. XI method of assay showed total alcohols, calculated as santalol, 91.5 per cent. While the U.S.P. XI does not assay the oil for esters, it was found by saponification to contain 1.9 per cent esters, calculated as santalyl acetate. Correcting for the esters, the free santalol content of the oil was 89.6 per cent.

The results of the analyses of these alcohols and volatile oils with the various reagents and different periods of heating, both on the steam bath and over an open flame, are given in Tables 1 to 6.

TABLE 1.—*Recovery of l-menthol*

REAGENT	WEIGHT SAMPLE	HEATING TIME, STEAM BATH	MENTHOL	WEIGHT SAMPLE	HEATING TIME FLAME	MENTHOL
	<i>gram</i>	<i>hours</i>	<i>per cent</i>	<i>gram</i>	<i>hours</i>	<i>per cent</i>
A	0.4739	0.5	89.1	0.4758	0.5	96.3
	0.4724	1.0	97.4	0.5068	1.0	99.1
	0.4915	1.5	99.0	0.5238	1.5	99.9
	0.5164	2.0	98.9	0.5247	2.0	99.4
B	0.4964	0.5	70.9	0.4939	0.5	94.4
	0.5038	1.0	88.5	0.4860	1.0	99.8
	0.4913	1.5	94.9	—	—	—
	0.4854	2.0	98.4	0.4992	2.0	99.8
C	0.4973	0.5	98.3	0.4963	0.5	99.6
	0.5070	1.0	99.0	0.5093	1.0	99.0

TABLE 2.—*Recovery of d-neomenthol*

REAGENT	WEIGHT SAMPLE	HEATING TIME, STEAM BATH	d-NEOMENTHOL	WEIGHT SAMPLE	HEATING TIME, FLAME	d-NEOMENTHOL
	<i>gram</i>	<i>hours</i>	<i>per cent</i>	<i>gram</i>	<i>hours</i>	<i>per cent</i>
A	0.5221	0.5	29.9	0.5082	0.5	55.9
	0.5021	1.0	49.5	0.5057	1.0	77.1
	0.5118	1.5	61.3	0.5123	1.5	84.0
	0.5084	2.0	70.3	0.4930	2.0	91.1
	—	—	—	0.4969	2.5	91.6
	—	—	—	0.5090	3.0	93.7
B	0.5129	0.5	25.0	0.5072	0.5	71.4
	0.4991	1.0	41.3	0.5155	1.0	90.4
	0.5208	1.5	54.1	0.5021	1.5	96.6
	0.5067	2.0	63.8	0.5034	2.0	98.0
C	0.4930	0.5	28.0	0.4981	0.5	62.9
	0.4993	1.0	50.7	0.5102	1.0	82.3
	0.5010	1.5	66.1	0.5003	1.5	92.6
	0.4878	2.0	75.9	0.4899	2.0	96.4

## DISCUSSION OF RESULTS

The various reagents were used on the alcohols to determine the conditions by which they could be quantitatively recovered. In the case of *l*-menthol, 1–2 hours' boiling with a flame was sufficient to recover 99.0 per cent or more with all three reagents. One hour on the steam bath with reagent C also gave 99.0 per cent recovery, while it required 2 hours for reagent A to give this yield. Hall, Holcomb, and Griffin (4) found that all the isomers of menthol studied by them could be acetylated with acetic



TABLE 3.—*Assay of oil of peppermint\**

REAGENT	WEIGHT SAMPLE	HEATING TIME, STEAM BATH	FREE MENTHOL	WEIGHT SAMPLE	HEATING TIME, FLAME	FREE MENTHOL
	<i>grams</i>	<i>hours</i>	<i>per cent</i>	<i>grams</i>	<i>hours</i>	<i>per cent</i>
A	—	—	—	1.0056	1.0	46.7
	1.0045	1.5	47.0	1.0281	1.5	47.3
	0.9936	1.5	46.4	1.0004	1.5	47.1
	1.0115	2.0	47.5	0.9903	2.0	47.1
	0.9962	2.0	46.8	1.0084	2.0	47.6
B	0.9914	1.0	41.8	0.9947	1.0	49.2
	—	—	—	0.9942	1.5	49.2
	1.0164	2.0	46.3	0.9827	2.0	49.5
C	1.0497	1.0	46.9	1.1308	0.5	47.4
	.9932	1.5	47.1	1.0412	1.0	48.6
	.9679	2.0	48.2	1.0201	2.0	49.1

\* 50.2% free menthol by U.S.P. XI assay.

TABLE 4.—*Recovery of borneol*

REAGENT	WEIGHT SAMPLE	HEATING TIME, STEAM BATH	BORNEOL	WEIGHT SAMPLE	HEATING TIME, FLAME	BORNEOL
	<i>gram</i>	<i>hours</i>	<i>per cent</i>	<i>gram</i>	<i>hours</i>	<i>per cent</i>
A	0.5065	0.5	78.7	0.4989	0.5	91.6
	0.5007	1.0	92.1	0.4982	1.0	97.7
	0.5045	1.5	96.4	0.5071	1.5	97.8
	0.5128	2.0	96.1	0.5060	2.0	98.6
	—	—	—	0.5132	2.0	99.0
B	0.5014	0.5	71.8	0.5055	0.5	97.2
	0.5030	1.0	87.8	0.5021	1.0	99.7
	0.5092	1.5	94.5	0.5038	1.0	99.8
	0.5073	2.0	97.9	0.5075	2.0	99.7
C	0.5018	0.5	84.8	0.5050	0.5	98.2
	0.5027	1.0	95.6	0.4974	1.0	99.7
	0.4988	1.5	96.6	—	—	—
	0.5017	2.0	98.7	0.4891	2.0	99.6

anhydride and sodium acetate in 1 hour with one exception, *d*-neomenthol, which required 2 hours for complete acetylation. The results on *d*-neomenthol obtained with reagents A, B, and C confirm these findings and show that *d*-neomenthol acetylates very slowly; reagent B in this case gave the best recovery, 98.0 per cent after 2 hours' boiling. Whether there is any *d*-neomenthol present in American peppermint oil is not known; however, Pickard and Littlebury (19) obtained a small amount from Japanese peppermint oil.

TABLE 5.—*Assay of oil of rosemary\**

REAGENT	WEIGHT SAMPLE	HEATING TIME, STEAM BATH	FREE BORNEOL	WEIGHT SAMPLE	HEATING TIME, FLAME	FREE BORNEOL
	<i>grams</i>	<i>hours</i>	<i>per cent</i>	<i>grams</i>	<i>hours</i>	<i>per cent</i>
A	—	—	—	4.0823	1.0	7.9
	4.2127	2.0	7.8	4.1261	2.0	8.2
B	—	—	—	4.3347	0.5	8.2
	—	—	—	3.9763	1.0	8.7
	—	—	—	3.9249	2.0	9.3
C	—	—	—	3.9524	1.0	8.0
	—	—	—	3.8477	1.5	8.2
	3.8491	2.0	8.0	4.0031	2.0	8.4

\* 11.8% free borneol by U.S.P. XI assay.

TABLE 6.—*Assay of oil of santalwood\**

REAGENT	WEIGHT SAMPLE	HEATING TIME, STEAM BATH	FREE SANTALOL	WEIGHT SAMPLE	HEATING TIME, FLAME	FREE SANTALOL
	<i>grams</i>	<i>hours</i>	<i>per cent</i>	<i>grams</i>	<i>hours</i>	<i>per cent</i>
A	1.0217	0.5	82.1	1.2636	0.5	82.3
	-1.0063	1.0	82.3	1.1278	1.0	82.5
	0.9969	1.5	82.4	0.9959	1.5	83.4
	0.9904	2.0	82.7	1.0061	2.0	83.8
B	1.0268	0.5	82.6	0.9963	0.5	84.3
	0.9996	1.0	83.9	0.9900	1.0	83.9
	0.9903	1.5	82.7	1.0208	1.5	84.3
	0.9975	2.0	83.1	0.9926	2.0	84.1
C	1.0127	0.5	81.5	0.9964	0.5	82.2
	1.0102	1.0	82.0	0.9995	1.0	82.2
	1.0110	1.5	82.1	1.0093	1.5	82.0
	0.9927	2.0	82.0	1.0054	2.0	82.4

\* 89.6% free santalol by U.S.P. XI assay.

The question now arises as to which of the methods tried is the best for the determination of menthol in oil of peppermint. It must be realized that there is no specific reagent that will determine primary, secondary, or tertiary alcohols in volatile oils, and nothing else. Therefore, the reagent and conditions chosen should determine the maximum amount of the alcohols and a minimum of other constituents such as aldehydes, unsaturated hydrocarbons, etc. To attain this goal, a quantitative determination of every constituent in the volatile oil would be necessary and each constituent would have to be treated with the various acetylating agents to find the conditions for its maximum or minimum reaction. This, ob-

viously, would constitute a major undertaking. Until this is done, a method must be selected which, from the information now available, is believed to give the best answer. The U.S.P. XI method, because of its more drastic treatment, is believed to give more undesirable side reactions than the methods discussed in this paper. The results obtained on oil of peppermint with reagents A, B, and C are approximately 2-6 per cent lower than the calculated free menthol by the U.S.P. XI method. The analyst can almost take his choice of the three reagents used; however, it is believed that 1-1.5 hours' refluxing with a flame and the use of reagent B would be most desirable for the assay of oil of peppermint. This reagent gives somewhat higher results than does A or C, which might be due to the presence in the oil of some of the more slowly reacting *d*-neomenthol or possibly some tertiary alcohol, the xylene acting as an indifferent medium according to the idea of Boulez (18).

The results show that borneol can also be recovered quantitatively by all three reagents, B and C requiring less time and giving more complete recovery than A. In the case of oil of rosemary, it is obvious that there is some constituent of an unknown nature, which is slowly acetylating. However, it is known that borneol itself can be completely recovered by reagents B and C and it is recommended that the oil be assayed by reagent B, and that a heating period of 1-1.5 hours with the flame be used. The results obtained by this method may be 20-30 per cent lower than those obtained by the U.S.P. XI method, but they should more nearly represent the actual amount of borneol present.

The results on oil of sandalwood with the three reagents are approximately 7-9 per cent lower than those obtained by the U.S.P. XI method; nevertheless, the writer believes that they represent a more accurate estimate of the santalol content of the oil, an opinion also shared by Delaby and Breugnot (1). Reagent B, heated for 1-1.5 hours with the flame, is also suggested for the assay of oil of sandalwood. It should be pointed out that the U.S.P. XI has made no provision for determining esters in oil of sandalwood, thus introducing an error in the determination of santalol by this method.

When the methods with reagents A, B, and C are used, an acid content of the oil should always be determined and, if excessive, a correction made.

#### SUMMARY

Criticisms of the U.S.P. XI assays for oils of peppermint, rosemary, and sandalwood and a literature review of other methods have been presented.

The Smith-Bryant (16) pyridine-acetyl chloride method, the Verley-Bolsing (5) pyridine-acetic anhydride method, the Radcliffe-Chadderton (12) pyridine-phthalic anhydride method as modified by Sabetay (15),

and a pyridine-acetic anhydride-xylene method have been investigated. The last three methods were found to be the most promising and to give results of the same order.

It has been recommended that the pyridine-acetic anhydride-xylene reagent with a 1-1.5 hour heating period over a flame be used for the assay of these three oils. In addition to allowing the direct determination of the free alcohols, this method is more convenient and more rapid than the U.S.P. XI assay, and the results probably represent more nearly the true alcohol content of the oils.

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#### THE INFLUENCE OF CERTAIN FACTORS ON REMOVAL OF FLUORINE FROM SUPERPHOSPHATES\*

By W. H. MACINTIRE, L. J. HARDIN, and J. W. HAMMOND

For many years it has been recognized that substantial decrease in  $P_2O_5$  availability may occur when superphosphates are conditioned with high-calcic limestone. This same deleterious effect has now become a problem in the manufacture of ammoniated fertilizers, and its cause has been the subject of investigation in both institutional and commercial laboratories.

The differential behavior of limestone and dolomite in their mixtures with superphosphates was reported from the Tennessee Agricultural Experiment Station (8, 9, 10, 11, 14), and reactions of dolomite were studied by Beeson and Ross (2) and by Keenen and Morgan (7). The effect of

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\* A cooperative study conducted at The University of Tennessee Agricultural Experiment Station under auspices of the Tennessee Valley Authority, Department of Chemical Engineering.

degree of ammoniation and the function of component calcium sulfate in subsequent reversion reactions were shown by Keenen and others (3, 4, 5, 6). Ross and associates (18) attributed degradation in  $P_2O_5$  availability in ammoniated superphosphates to the formation of hydroxy tricalcium phosphate, or hydroxy apatite.

An exceptional degree of  $P_2O_5$  retrogradation (14) was encountered by the present authors in certain studies as to the behavior of mixtures of superphosphates and calcium silicate slag of relatively high fluoride content. This experience led to the postulation that the retrogradation had been caused by the formation of calcium fluorophosphate,  $Ca_{10}(PO_4)_6 \cdot F_2$ , through reaction between generated tricalcium phosphate and the calcium fluoride of the slag (14, 16). Further study demonstrated that the fluoride components of superphosphates were ample to cause substantial  $P_2O_5$  retrogradation in mixtures with fluoride-free calcic materials and also upon ammoniation (16). The postulation was substantiated by the finding that no reversion occurred when superphosphates of meager fluoride content were mixed with fluoride-free liming materials, or when such superphosphates were ammoniated (17).

Since component fluorides (a) induce development of citrate-insoluble  $P_2O_5$  in limed and in ammoniated superphosphates, (b) are detrimental in mixtures of limestone and superphosphates for livestock, and (c) constitute a potential source of by-product fluorides, the desirability of superphosphates of low fluorine content is obvious. The present studies were planned, therefore, primarily to determine to what extent moderate heat treatments would effect removal of fluorine from concentrated superphosphate without detriment to  $P_2O_5$  availability. Percentage removal of fluorine was the criterion of the effectiveness of a given treatment. Additional considerations were the resultant (a) increase in  $P_2O_5$  percentage, (b) changes in  $P_2O_5$  availability, and (c) development of metaphosphates in the heat-dehydrated superphosphates.

#### LOSS OF FLUORINE DURING ACIDULATION

The evolution of fluorine during acidulation accounts for only a fraction of that present in the raw rock phosphate, and is greater for acidulation with  $H_2SO_4$  than for acidulation with  $H_3PO_4$ . Removal of fluorine during the manufacture of superphosphates is governed by the extent of the reaction of the added acid upon the calcium fluoride split off from the apatite molecule and attendant formation of  $HF$  and  $SiF_4$ . The removal of fluorine from the acidulated mass is not effected readily, even when the added acid is in excess of the equivalence of the total calcium content of the raw rock.

#### EXPERIMENTAL

The superphosphates used in the present study were made by acidulation of raw and processed rocks with  $H_3PO_4$ , or with a mixture of  $H_2PO_4$

and  $\text{H}_2\text{SO}_4$ , and variant equivalences of acid were used. Heat treatment of the superphosphates was employed, either alone or as one step in the conditions imposed to accelerate formation and expulsion of volatile fluorine compounds. Supplements of chlorides, silica, and borates were used under various conditions to induce secondary reactions and the formation of more volatile fluorides. The fluorine determinations were made by the perchloric acid distillation method of Willard and Winter (19).

The  $\text{P}_2\text{O}_5$  analyses were made titrimetrically according to A.O.A.C. methods (1). Total water-soluble  $\text{P}_2\text{O}_5$  was determined by molybdate precipitations in leachate aliquots that had been boiled 10 minutes with an excess of 5 ml. of  $\text{HNO}_3$ . The differences between the  $\text{P}_2\text{O}_5$  found in the  $\text{HNO}_3$ -digested aliquots of the aqueous extracts and that found in the ortho form in corresponding undigested aliquots represented the  $\text{P}_2\text{O}_5$ -equivalences of the  $\text{PO}_3$  of the water-extractable metaphosphates generated in the superphosphates during the heat treatments (15).

*Effects Induced by Heating Superphosphates at Different Temperatures, With and Without Additive Materials*

A number of preliminary heating trials at high temperatures were conducted. Four phosphatic materials—a monocalcium phosphate, a superphosphate of meager fluoride content, and cured commercial superphosphates from Florida and Tennessee rocks—were mixed with either potassium chloride, magnesium chloride, magnesium sulfate, or selectively calcined dolomite, and the resultant mixtures heated 10 minutes at  $300^\circ\text{C}$ , and at  $600^\circ\text{C}$ . In all, 59 conditions were imposed.

Heated 10 minutes at  $600^\circ\text{C}$ ., superphosphates derived from Florida and Tennessee rocks suffered respective fluorine losses of 75 per cent and 68 per cent; but only meager removals occurred in the dry mixtures that contained the selectively calcined dolomite. In general, complete conversion of ortho phosphates to water-insoluble metaphosphates occurred, and citrate-insoluble  $\text{P}_2\text{O}_5$  content was increased threefold.

The averages of fluorine removals by the  $300^\circ\text{C}$ . heating of the Florida and Tennessee superphosphate mixtures were 56 and 44 per cent, respectively. The added salts diminished the expulsion of fluorine, and the diminution effected by the high iron and alumina content of the Tennessee rock was particularly evident. The  $300^\circ\text{C}$ . treatment converted approximately 50 per cent of the water-soluble ortho phosphates to water-soluble meta forms, and practically no development of citrate-insolubility occurred, except in the mixtures that contained selectively calcined dolomite.

The  $\text{P}_2\text{O}_5$  transitions induced in the fluoride-free mixtures at both  $200^\circ\text{C}$ . and  $600^\circ\text{C}$ . were similar to those induced by the same heat treatments of the corresponding mixtures that contained fluorides.

Since the imposition of the two high temperatures resulted in detrimental  $\text{P}_2\text{O}_5$  transformations in some instances, lower temperatures and

longer periods were imposed. Fluorine removal was small when well-cured Tennessee and Florida rock superphosphates of 1 to 2 per cent moisture content were heated for 10 minutes at 150° C.; practically no development of either metaphosphate or citrate-insoluble  $P_2O_5$  occurred and the concentration of  $P_2O_5$  content was only 1 to 2 per cent. When green superphosphates were heated 30 minutes at 150° C., however, about one-third of the fluorine was removed, appreciable transitions to metaphosphate developed, and some reversion occurred. None of these changes was induced to appreciable extent when green superphosphates were heated 30 to 40 minutes at 65° C.

It was established also that the removal of fluorine by the 150° C. and 200° C. immediate heatings of experimental superphosphates that had been granulated during acidulation, exceeded the removals from corresponding superphosphates that had been allowed to solidify into a dense mass before imposition of similar heat treatments.

Because of these observations, fresh granular experimental superphosphates only were heated thereafter, except those of Table 1. The subsequent heatings were at either 150° C. or 200° C. for 30 minutes or longer in a ventilated furnace, and without additive materials other than silica and borates.

#### *Effect of Degree of Acidulation on the Removal of Fluorine by Heating Superphosphates*

Four distinct types of rock phosphate were acidulated with 78 per cent  $H_3PO_4$ , equivalent to 82 per cent of calcium + iron + aluminum,\* and also upon 100 per cent equivalence of calcium content. Each resultant superphosphate then was passed through a 12-mesh sieve and a portion heated immediately at 200° C. for 40 minutes. Another portion was cured 3 weeks at room temperature and then heated likewise. The changes that ensued during the heat treatments are shown in Table 1.

The  $H_3PO_4$  requirement for the acidulation of high-grade rock, upon the basis of calcium content, was about 12 per cent more than that required by 82 per cent of the equivalence of Ca + I and A. The superphosphates obtained from high-grade rock by the 100 per cent acid equivalence of calcium content contained more initial free  $H_3PO_4$  than did those by the acidulations of 82 per cent of the joint equivalence of calcium and I and A. The heat treatment induced greater removals of fluorine from the superphosphates made by acidulation on the 100 per cent basis.

The fluorine removals induced by the immediate heating of the several fresh superphosphates exceeded by  $\frac{1}{3}$  to 5-fold those induced by heating after curing. The heat treatment diminished the free acid content of most of the superphosphates to nugatory percentage. Slight increases in citrate-

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\* Referred to as "I and A" throughout the present text.

TABLE 1.—*Effects of heating<sup>a</sup> superphosphates made from four types of Tennessee phosphate rock by two degrees of acidulation*

ROCK TYPE	ACIDULATION		P <sub>2</sub> O <sub>5</sub> PERCENTAGE ANALYSIS OF HEATED EXPERIMENTAL SUPERPHOSPHATES									
	EQUIVALENCE OF H <sub>3</sub> PO <sub>4</sub> %	RATIO OF ACID IN 82% AND 100% ACIDULATION	FREE ACID		C. I. INCREASE DUE TO HEATING	FRACTION OF W. S. IN META FORM IN HEATED PRODUCT	BY IMMEDIATE HEATING	BY HEATING AFTER 3 WEEKS CURING	CONCENTRATION BY IMMEDIATE HEATING			
			BEFORE HEATING	AFTER HEATING								
Tenn. brown <sup>b</sup>	82 <sup>f</sup> 100 <sup>g</sup>	1:1.125	5.93 7.89	0.14 0.10	0.25 0.20	49 46	57 61	14 20	15.7 16.4			
Low grade matrix <sup>c</sup>	82 <sup>f</sup> 100 <sup>g</sup>	1:0.789	5.60 7.24	0.21 0.21	1.00 2.00	35 47	3 11	— 2	16.9 15.5			
High grade washed sands <sup>d</sup>	82 <sup>f</sup> 100 <sup>g</sup>	1:1.08	3.48 4.63	0.28 0.36	0.80 0.50	65 50	52 50	32 38	19.0 17.0			
Tenn. blue <sup>e</sup>	82 <sup>f</sup> 100 <sup>g</sup>	1:1.15	3.91 5.05	1.42 0.86	0.90 0.70	37 26	55 60	43 55	14.4 13.3			

<sup>a</sup> 12-mesh product heated 40 minutes at 200° C.<sup>b</sup> Sample No. 8704 containing 66.2 % B.F.L., 574 % "I and A," 6.46 % CaF<sub>2</sub>, and 2.70 % CaCO<sub>3</sub>≡ of CO<sub>2</sub>.<sup>c</sup> Sample No. 8717 containing 40.2 % B.F.L., 19.58 % "I and A," 3.52 % CaF<sub>2</sub>, and 0.90 % CaCO<sub>3</sub>≡ of CO<sub>2</sub>.<sup>d</sup> Sample No. 8716 containing 81.72 % B.F.L., 2.46 % "I and A," 8.00 % CaF<sub>2</sub>, and 1.50 % CaCO<sub>3</sub>≡ of CO<sub>2</sub>.<sup>e</sup> Sample No. 8718 containing 60.10 % B.F.L., 3.77 % "I and A," 5.97 % CaF<sub>2</sub>, and 2.54 % CaCO<sub>3</sub>≡ of CO<sub>2</sub>.<sup>f</sup> Basis of calcium + I and A content.<sup>g</sup> Basis of calcium content.



TABLE 2.—Removal of fluorine from "synthetic" apatite<sup>a</sup> during acidulation<sup>b</sup> and subsequent heating at 150° C.

PROPERTIES AND PERCENTAGE ANALYSES OF EXPERIMENTAL SUPERPHOSPHATES										
TREATMENT OF MIXTURES PRIOR TO ACIDULATION <sup>c</sup>		PHYSICAL CONDITION <sup>b</sup>	CURING PERIOD PRIOR TO HEATING	CONCENTRATION BY HEATING AT 150° C.		FLUORINE				
TEMP. °C.	MOISTURE CONDITION			40 MIN.	60 MIN.	PRIOR TO HEATING	HEATED 40 MIN.		HEATED 60 MIN.	
							FOUND <sup>d</sup>	REMOVAL %	FOUND <sup>d</sup>	REMOVAL %
<i>From mixtures without iron<sup>d</sup></i>										
25	dry	dry lumps	4 hours	12.5	14.7	1.18	0.98	17	0.93	21
25	moist <sup>f</sup>	damp, gummy	5 days	11.4	12.8	1.10	0.96	13	0.97	12
65	dry	dry, gummy	4 hours	10.6	14.3	1.19	1.03	13	0.94	21
65	moist <sup>f</sup>	thick slurry	20 hours	12.9	16.0	1.13	0.82	27	0.71	37
<i>From mixtures supplemented with precipitated Fe<sub>2</sub>O<sub>3</sub> · 2H<sub>2</sub>O<sup>g,h</sup></i>										
25	dry	dry lumps	4 hours	12.3	13.7	1.19	1.05	12	1.06	10
25	moist <sup>f</sup>	damp, gummy	4 days	10.1	10.7	1.28	1.10	15	1.09	15
65	dry	dry, gummy	6 hours	11.0	12.9	1.19	1.06	10	1.07	10
65	moist <sup>f</sup>	thick slurry	4 days	8.7	10.6	1.26	1.08	14	1.03	18

<sup>a</sup> Mixtures of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> + CAF<sub>3</sub> and also Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> + CAF<sub>3</sub> + Fe<sub>2</sub>O<sub>3</sub>.<sup>b</sup> Acidulation with 78% H<sub>3</sub>PO<sub>4</sub> based on CaO content of mixtures.<sup>c</sup> All mixtures were aged one week under conditions given.<sup>d</sup> Analysis of unacidulated mixture: CaO 52.2%, total P<sub>2</sub>O<sub>5</sub> 37.15%, fluorine 3.72%.<sup>e</sup> Analysis of unacidulated mixture: CaO 47.9%, total P<sub>2</sub>O<sub>5</sub> 34.60%, fluorine 3.65%, Fe<sub>2</sub>O<sub>3</sub> 8.3%.<sup>f</sup> Moisture brought to 40%, giving a damp lumpy mixture.<sup>g</sup> Corrected to initial weight basis.<sup>h</sup> The superphosphates from this mixture were initially reddish-brown. This color disappeared on heating 40–60 min at 150° C. It did not disappear, however, upon heating at 45° C. for 20 hours, but it did disappear after 24 hours at 45° C.

insoluble  $P_2O_5$  occurred during the heating of superphosphates derived from ordinary brown Tennessee rock. This effect was offset, however, by the dehydration which raised the available  $P_2O_5$  content to nearly 60 per cent. The difference in type of rock and the variation in acidulation exerted no significant effect upon the conversion of orthophosphates to metaphosphates. In general, about half of the water-soluble fraction of the heated superphosphates had been converted to water-soluble meta forms.

Increases up to 2 per cent in citrate-insoluble  $P_2O_5$  occurred, however, during the heating of superphosphates made from the low-grade argillaceous matrix. Certain observations should be made in connection with this material. It contained 19.58 per cent of I and A, and the  $H_3PO_4$  requirement for the 100 per cent equivalence of its total calcium content, therefore, was 20 per cent less than the acid requirement for the 82 per cent equivalence of its content of calcium +I and A.

The fluorine removals from the immediately heated superphosphates of unusually high I and A content were decidedly lower than those from the other heated products, and were almost negligible when these superphosphates were cured 3 weeks before heating. Increase in citrate-insoluble  $P_2O_5$  was much greater in the heated products from the cured superphosphates of high I and A content. The earlier heat treatments at 300° C. had effected greater fluorine removal from the Florida rock superphosphates than from the superphosphates derived from Tennessee brown rock. The dual effect was attributed to some neutralization of the  $H_3PO_4$  by the high I and A content of the brown rock. Since this I and A content had exerted such significant repression upon the removal of fluorine by heating the superphosphates, the four raw rocks were ignited 1 hour at 1000° C. prior to acidulations otherwise identical to those that had been made with the raw rocks. Such ignition caused no decrease in the repressive effect of the I and A upon the release of fluorine during the moderate heating of the superphosphates derived from the ignited rocks.

*Effect of  $Fe_2O_3$  on Fluorine Removal from "Synthetic" Apatite  
During Acidulation and Subsequent Heating*

The repressive effect of iron content upon fluorine release during heat treatment was studied further by means of the eight experimental superphosphates made from the "synthetic" fluorophosphate materials of Table 2. One series comprised superphosphates derived from dry mixtures of laboratory-prepared tricalcium phosphate and calcium fluoride, and superphosphates derived from the similar mixtures that had been aged moist one week at 65° C. The other series comprised superphosphates made from corresponding mixtures supplemented with 8.3 per cent of  $Fe_2O_3$ , in the form of colloidal hydrated iron oxide. All acidulations were upon the basis of calcium content.

The green superphosphates of the two series were heated at 150° C.

for periods of 40 and 60 minutes. The fluorine removals from the no-iron series and the series fortified with iron oxide averaged 23 and 13 per cent, respectively, for the 60-minute heating period. These results and others demonstrate that the presence of  $\text{Fe}_2\text{O}_3$  exerts a repressive effect upon fluorine removal by heat treatment of both fresh and cured superphosphates. This effect may be due to both physical and chemical factors. It is probable that a repressive effect upon fluorine removal was induced also by the  $\text{Al}_2\text{O}_3$  content of the brown rock matrix, used earlier as a source of the superphosphates that showed no appreciable loss of fluorine upon heating.

*Effect of Silica Supplements on the Removal of Fluorine from Superphosphates During Heating*

Reaction between silica and the HF generated during acidulation results in the evolution of silicon tetrafluoride. Different forms of silica vary,

TABLE 3.—*Removal of fluorine from heated fresh superphosphates derived from mixtures of brown rock and silica<sup>a</sup>*

SILICA SUPPLEMENT		HEATING TIME AT 200° C., MIN.	PERCENTAGE ANALYSIS OF HEATED SUPERPHOSPHATES			
			$\text{P}_2\text{O}_5$			FLUORINE REMOVAL <sup>d</sup>
			TOTAL	CIT.-INSOL. INCREASED <sup>d</sup>	FRACTION OF W.S. IN META FORM	
None	—	40	57.8	0.53	34	55
None	—	60	58.5	0.42	42	60
Tripoli <sup>b</sup>	5	40	55.6	0.32	23	57
Tripoli	5	60	58.0	0.50	43	60
Tripoli	10	40	54.7	0.40	28	58
Tripoli	10	60	55.6	0.42	37	61
Precipitated <sup>c</sup>	5	40	57.3	0.52	26	66
Precipitated	5	60	58.1	0.50	42	66
Precipitated	10	40	55.4	0.14	30	62
Precipitated	10	60	57.5	0.21	42	64
Quartz powder	5	40	58.4	0.11	36	65
Quartz powder	5	60	59.8	0.08	51	74
Quartz powder	10	40	54.2	0.18	27	65
Quartz powder	10	60	56.5	0.35	43	71

<sup>a</sup> By acidulation with 78%  $\text{H}_3\text{PO}_4$  equivalent to 82% of calcium + I and A.

<sup>b</sup> A natural powdery product.

<sup>c</sup> Obtained from wash towers of a commercial plant; after washing with 1% HCl, the air-dried material contained 1.5 per cent of fluorine and 10 per cent of water of hydration.

<sup>d</sup> By comparison with the green superphosphate.

however, in their effects upon the release of fluorine from acidic phosphatic systems under laboratory conditions. To determine the effect of

variation in form and amount of additive silica upon removal of fluorine during heat treatment of superphosphates, mixtures of brown rock and 5 and 10 per cent additions of silica were acidulated with 78 per cent  $\text{H}_3\text{PO}_4$  upon the basis of calcium + I and A. Three forms of silica—tripoli, precipitated silicic acid, and powdered quartz—were used. The fresh silica-fortified superphosphates were heated at  $200^\circ\text{C}$ . for 40 and 60 minutes.

The data of Table 3 show that the supplements of tripoli, silicic acid, and quartz induced respective enhancements of 2, 8, and 12 per cent in fluorine removal. A mean fluorine loss of 64 per cent occurred in the heated silica-supplemented superphosphates against a loss of 57 per cent from the unsupplemented controls. The 60-minute heat treatment induced, on the average, a removal 40 per cent greater than that for the 40-minute treatment. The maximal fluorine removal of 74 per cent was obtained by heating the quartz-supplemented superphosphate and the dehydrated product contained only 0.39 per cent of fluorine. Silica additions of 5 per cent seemed to be as efficacious as those of 10 per cent in promoting fluorine removal.

The mean increase in citrate-insoluble  $\text{P}_2\text{O}_5$  in the heated products from the silica-supplemented superphosphates was only 0.33 per cent, which was somewhat less than that in the heated products from the straight superphosphates. The heat treatment caused a mean net increase of 7.7 per cent in available  $\text{P}_2\text{O}_5$ . More than a third of the water-soluble  $\text{P}_2\text{O}_5$  content of the heated products had been converted to the meta form, and a slightly higher percentage conversion was induced by the longer heating.

*Effect of Heat on Superphosphates Obtained by  
Acidulations with  $\text{H}_3\text{PO}_4 + \text{H}_2\text{SO}_4$*

The effect of a 10 per cent substitution of  $\text{H}_2\text{SO}_4$  for  $\text{H}_3\text{PO}_4$  upon fluorine removal during acidulation and subsequent heating was determined. A superphosphate was made from brown rock by a 100 per cent calcium-equivalence acidulation with a 90 to 10 mixture of 78 per cent  $\text{H}_3\text{PO}_4$  and 66 per cent  $\text{H}_2\text{SO}_4$ . Portions of this superphosphate were heated at  $150^\circ\text{C}$ . for 10 minutes and for 20 and 40 minutes, with stirring at 10-minute intervals and without stirring. The heated products showed fluorine removals proportional to duration of heating, and slightly higher removals occurred in the stirred mixtures. One portion, heated 120 minutes without stirring, lost 78 per cent of its fluorine content and gained 12 per cent in available  $\text{P}_2\text{O}_5$ . Approximately one-half of the water-soluble  $\text{P}_2\text{O}_5$  content of the heated superphosphate was in the meta form. In every case, the citrate-insoluble  $\text{P}_2\text{O}_5$  of the heated superphosphate was less than that of the unheated material. All of the heated superphosphates of the  $\text{H}_3\text{PO}_4 + \text{H}_2\text{SO}_4$  acidulations were in good condition at the end of the heating period, but they showed deliquescence upon exposure after grinding. This deliquescence cannot be attributed to generated metaphosphates, since

the  $P_2O_5$  transitions induced during the heat treatment of the superphosphates from  $H_3PO_4 + H_2SO_4$  acidulations were less than those induced by similar heating of straight superphosphates.

*Effect of Ignition of Phosphate Rock in Steam  
Atmosphere Prior to Acidulation*

The fluorine removal from the 200° C. heating of superphosphates derived from the 600° C. and 900° C. incinerates of rock phosphate in air was comparable to the removal from superphosphates derived from the unignited rock phosphates. It was considered probable, however, that ignition at 1000° C. in a total atmosphere of steam might induce removal of fluorine from the raw rock and also affect the reactivity of its fluoride and I and A content during subsequent acidulation. When brown phosphate rock was heated one hour at that temperature in an atmosphere of steam, it lost 43 per cent of its fluorine content and was bleached to an ivory tint. When the same rock was supplemented with 10 per cent of precipitated silicic acid and heated at 1000° C. in an atmosphere of steam, it lost 52 per cent of its fluorine content. Upon acidulation with 78 per cent  $H_3PO_4$  upon the basis of calcium + I and A, the incinerated rock was decomposed about one-fifth as rapidly as raw rock and yielded a white superphosphate.

The aggregate of fluorine removal (Table 4) induced jointly by the prior ignition of the rock in the steam atmosphere and the heating of the derivative superphosphates, however, amounted to 80 per cent of the fluorine content of the raw rock. Apparently, the beneficial effects of the preliminary processing with additive silica did not extend into the subsequent steps of acidulation and heating. The product from the 40-minute heating of the superphosphate by 100 per cent acidulation of the rock-silica mixture heated at 1000° C. in an atmosphere of steam contained only 0.33 per cent of fluorine. It suffered no decrease in  $P_2O_5$  availability and more than half of its water-soluble  $P_2O_5$  content was in the meta form.

*Behavior of Superphosphates Obtained by  
Acidulation with 70 Per Cent  $H_3PO_4$*

The effect of less concentrated  $H_3PO_4$  upon fluorine removal from hot and cold acidulations was also determined. Removal during the acidulation of brown rock, heated to 200° C. and acidulated immediately with an 82 per cent calcium + I and A equivalence of cold 70 per cent  $H_3PO_4$ , was less than the removal during corresponding acidulations of cold rock with 78 per cent cold acid. When brown rock was heated to 200° C. and acidulated immediately with 70 per cent  $H_3PO_4$ , the resultant superphosphate lost only one fourth of its fluorine content during heat treatment of 60 minutes at 200° C. This fluorine removal was also much less than that obtained by the heating of superphosphates from ordinary acidulations with 78 per cent  $H_3PO_4$ . The 70 per cent concentration of  $H_3PO_4$ , both hot

TABLE 4.—*Effect of ignition\* of Tennessee brown phosphate rock with and without silica supplements, upon removal of fluorine by heat treatments of superphosphates derived from the ignited rock*

IGNITED ROCK <sup>a</sup>		SUPER- PHOSPHATE HEATED AT 200° C. MIN.	PERCENTAGE ANALYSIS OF HEATED SUPERPHOSPHATE					
SILICA SUPPLE- MENT <sup>b</sup> %	ACIDULA- TION $\approx$ OF 78% H <sub>3</sub> PO <sub>4</sub> <sup>c</sup>		P <sub>2</sub> O <sub>5</sub>				FLUORINE	
			TOTAL	WATER-SOLUBLE		CITRATE- INSOLUBLE	FOUND	FRACTION LOST <sup>d</sup>
				ORTHO	META			
none	82	none	48.5	44.5	—	3.3	0.85	—
		40	55.4	39.2	11.0	3.5	0.45	53
		60	57.2	30.5	20.2	3.6	0.42	60
10	82	none	46.4	42.5	—	3.5	0.93	—
		40	53.4	30.0	15.5	5.0	0.48	55
		60	55.1	21.0	25.5	5.2	0.50	55
10	100	none	47.6	45.6	—	2.5	0.73	—
		40	54.9	35.5	11.5	2.5	0.33	61
		60	57.7	22.5	27.5	2.8	0.32	63

\* Ignition 1 hour at 1000° C. in an atmosphere of steam, with and without silica supplements, caused respective fluorine losses of 44 and 52 per cent.

<sup>b</sup> Precipitated silicic acid from the scrubbing tower of a superphosphate manufacturing plant; fluorine content of 1.5 per cent.

<sup>c</sup> Sum of B, P, L, CaF<sub>2</sub>, CaCO<sub>3</sub>, and I and A.

<sup>d</sup> In addition to the loss of approximately one half of the content of the raw rock during the preliminary ignition.

and cold, also proved less efficacious for fluorine removal in corresponding acidulations and similar heat treatments of several other combinations with hot rock.

### Miscellaneous Treatments

A prior treatment of the brown rock with H<sub>2</sub>O<sub>2</sub> of 30 per cent concentration had no appreciable effect upon fluorine removal, when the derivative superphosphate was heated at 200° C. for 60 minutes. Supplements of boron, as either sodium borate or boric acid, induced no increase in fluorine removal during either the acidulation of brown rock or the moderate heating of the derivative superphosphates.

A 75 per cent removal of fluorine was obtained, however, when a brown rock, supplemented with 5 per cent of SiO<sub>2</sub> and with a 20 per cent addition of an oxidative mixture containing equal parts of KClO<sub>3</sub> and MnO<sub>2</sub>, was acidulated with 78 per cent H<sub>3</sub>PO<sub>4</sub>, and the resultant superphosphate heated 60 minutes at 200° C.

### SUMMARY

Studies as to means for the removal of fluorine from superphosphates were prompted by recognition of (a) component fluorides as the dominant cause of the development of citrate-insoluble P<sub>2</sub>O<sub>5</sub> in limed and in ammoni-

ated superphosphates, (b) the utility of mixtures of limestone and superphosphates of low fluorine content for livestock feeding, and (c) the increase in the demand for fluorides.

The following factors were considered in a study of the effect of heat upon the removal of fluorine: (a) acid concentration and equivalence, (b) fresh and cured superphosphates, (c) temperature and duration of heat treatment, (d) granulation, (e) supplemental materials, (f) influence of iron, and (g) prior ignition of phosphate rock in air and in steam. Concomitant  $P_2O_5$  concentrations and transitions were determined.

Heat treatments at 600, 300, 200, and 150 degrees C. demonstrated that the almost complete removal of fluorine induced by the two higher temperatures was accompanied by extensive deleterious  $P_2O_5$  transformations.

When superphosphates were subjected to the preferred 200° C. heat treatment:

(a) Enhancement in fluorine removal was induced by extension of the heating period, and by stirring and granulation.

(b) No supplemental material, other than silica, accelerated fluorine removal.

(c) Iron content exerted a repressive effect upon the volatilization of fluorine.

(d) This repressive effect was not diminished in superphosphates derived from rock phosphates ignited in air.

(e) Almost no increase in citrate-insoluble  $P_2O_5$  occurred.

(f) As much as 50 per cent transition of the water-soluble ortho phosphates to water-soluble meta forms was induced.

The ignition of brown rock decreased the speed of acidulation.

Prior ignition of brown rock phosphate at 1000° C., in an atmosphere of steam also decreased reactivity during acidulation, caused a loss of more than 40 per cent of the fluorine content of the rock, and gave a bleached calcine that yielded a white superphosphate.

Acidulations with 78 per cent  $H_3PO_4$  were more effective than those with 70 per cent acid, regardless of temperature of rock and acid.

A 10 per cent replacement of  $H_3PO_4$  by  $H_2SO_4$  caused a deliquescence, when the heated superphosphates were ground and exposed.

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## DETERMINATION OF METALDEHYDE IN INSECTICIDES

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U. S. Department of Agriculture)

The employment of metaldehyde in insecticides used for the control of garden pests, such as slugs and sowbugs, necessitates the formulation of an accurate and simple method for its determination alone and also in the presence of carriers such as sawdust, bran, talc fillers, and arsenates.

The method here described has certain advantages in accuracy and economy of time over the technic used by Schonberg (1) although it involves the same principle, viz., the decomposition of the metaldehyde to acetaldehyde by mineral acid, the transfer of the acetaldehyde from the acid solution to bisulfite solution, and the titration of the "bound" sulfite with standard iodine solution. The "bound" sulfite gives a direct measure of the amount of acetaldehyde.

The acetaldehyde determination is a modification of the Rippert (2) method first used by Clausen (3), and later by Friedemann, Cotonio, and Shaffer (4), Tomoda (5), and other chemists (6) in the determination of homologous aldehydes. Excellent results were reported by Friedemann, Cotonio, and Shaffer, who ran recovery experiments on the purified acetaldehyde-bisulfite compound.

The transference of the acetaldehyde from the acid to the bisulfite solution offers the most difficulty. Schonberg and others suggest aeration as the means of transfer, and Friedemann, Cotonio, and Shaffer point out that aeration alone is not sufficient. The writer confirms the latter view, and his justification is shown in the recovery experiments. The procedure

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\* Resigned November 8, 1940.



that gave the best recoveries included aeration to remove most of the acetaldehyde, and steam distillation, which carried over the residual acetaldehyde into the bisulfite solution.

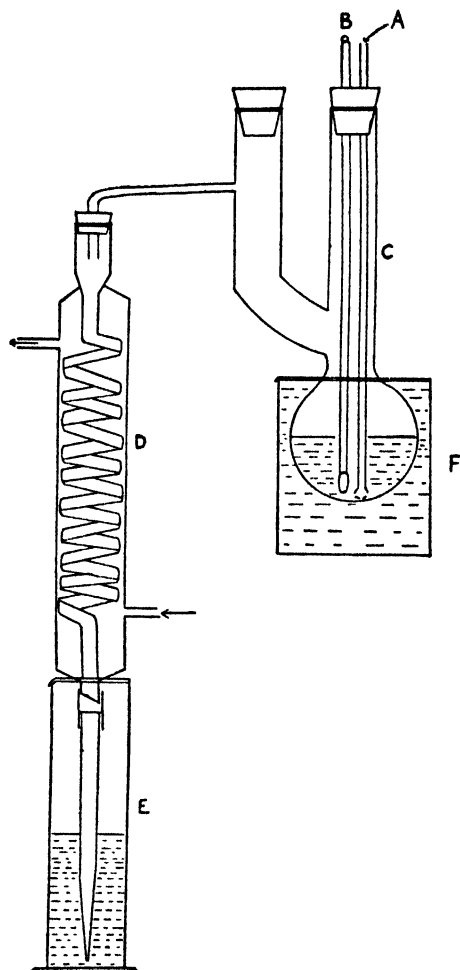


FIG. 1.—DISTILLATION APPARATUS.

A, air or steam inlet; B, thermometer; C, digestion flask; D, condenser; E, bisulfite receiver; F, water bath.

The method follows:

#### METHOD

##### REAGENTS

(a) *Normal sulfuric acid.*

(b) *Sodium bisulfite solution.*—Dissolve 25 grams of purified  $\text{Na}_2\text{S}_2\text{O}_3$  in water and make up to 1 liter.

(c) *Iodine solution.*—0.1 *N.* Dissolve 12.7 grams of I and 25.4 grams of KI in a minimum quantity of water and make up to 1 liter. Standardize the solution against standard thiosulfate solution.

(d) *Iodine solution.*—Approximately 1 *N.* Dissolve 63.5 grams of I and 127 grams of KI in a minimum quantity of water and make up to final volume of 500 ml.

(e) *Starch indicator.*—1% solution.

##### DETERMINATION

Place a sample containing approximately 0.1 gram of metaldehyde in a 150 ml. Claissen flask (see Figure 1), add 50 ml. of the  $\text{H}_2\text{SO}_4$ , and shake the mixture in order to get the material in contact with the acid. Stopper the main stem of the flask with a 2-holed rubber stopper, through which pass a thermometer and an air inlet tube, both reaching to near the bottom of the flask. Close the other opening with a solid stopper. Connect the side arm of the flask to a spiral condenser, and to the mouth of the condenser attach a glass tube tapered to a fine point, approximately 1 mm. in diameter, which dips into 40 ml. of the bisulfite solution, and extends close to the bottom

of the container, a tall glass cylinder. Connect the air supply to the air inlet tube, and so adjust the air current that approximately 4 bubbles of air per second pass through the bisulfite solution. (A good stream of cold water should be passing through the condenser before the air pressure is connected, in order to cool the air passing through the bisulfite solution, and the flask should be shaken at intervals if necessary to wet the material on the sides.) Heat the solution in the Claissen flask in a water bath to 60°–70° C. and keep at this temperature for about 1 hour. Then disconnect the air pressure and immediately attach a steam generator to the inlet tube by means of rubber tubing. Remove the water bath and distil 50

ml. over into the bisulfite solution. Make the distillate and bisulfite solution to a final volume of 200 ml. and use a 100 ml. aliquot for titration. Place the bisulfite solution in a 500 ml. Erlenmeyer flask, add 2-3 ml. of the starch indicator, titrate the excess of bisulfite by adding 5 ml. of the strong I solution, and finish the titration with the standard I solution to a blue-violet end point. Neutralize the solution with  $\text{NaHCO}_3$  powder and then add 5-10 grams in excess. When the solution becomes colorless, again titrate immediately with the standard solution to a blue-violet end point. The end point is reached when the color remains for 1 minute on the addition of 1 drop of the I solution.

1 ml. of 0.1  $N \text{ I}_2 = 0.0022$  gram of metaldehyde.

#### Notes

(1) When an aspirator is used in place of air pressure the receiver that contains the bisulfite should be attached to the spiral condenser by means of a 2-holed rubber stopper through which the glass tip passes into the bisulfite solution. A glass tube just reaching through the stopper connects with the aspirator.

(2) In the event of frothing during distillation, which may occur if organic matter such as sawdust or bran is present, a slowing down of the distillation will stop the frothing.

(3) The titration of the sulfite freed from the acetaldehyde-bisulfite compound should be carried out immediately since there is the possibility of the oxidation of the sulfite by air in the presence of a strong excess of bicarbonate, as pointed out by Clausen, and by Friedemann, Cotonio, and Shaffer.

(4) The addition of the iodine solution towards the end of the titration should be rather slow since the reaction between the iodine and the last traces of sulfite appears to be much slower than the initial reaction. However, the end point is quite distinct.

#### RECOVERY EXPERIMENTS

The results of recovery experiments are shown in Tables 1 and 2. For these experiments commercial metaldehyde was recrystallized out of absolute alcohol, filtered, washed with several portions of dry ether while on the filter, and then air dried.

#### DISCUSSION

When sulfuric acid (1+4) and aeration alone for 1 hour were used, the recovery of metaldehyde averaged 92.25 per cent (Table 1, Part I). The use of the Schonberg apparatus and technic gave no better results.

When sulfuric acid (1+4), aeration for the same length of time, and steam distillation were used, the recovery of metaldehyde averaged 98 per cent (runs 1 and 2, Table 1, Part II). For runs 3, 4, and 5, the end point was not distinct because of the presence of interfering substances as a result of the steam distillation. The average recovery for the last three runs is 100.58 per cent.

The high recoveries shown in Table 1, Part II, led to a study of blank determinations on the usual metaldehyde carriers. The same procedure was used. Arsenic, which may be an interfering substance, was not found present in the distillate. Table 2(A) shows that there is distilled over a substance that is capable of reacting with the bisulfite. In order to eliminate the blank several changes in the procedure were tried. The method that gave consistent recoveries and an inconsequential blank resulted

TABLE 1.—*Recovery of pure metaldehyde*

	WT. OF METALDEHYDE TAKEN	0.1 N I REACTING WITH "BOUND" SULFITE	METALDEHYDE RECOVERED	
	grams	ml.	grams	per cent
PART I*				
1	0.0491	20.41	0.0449	91.5
2	0.0500	20.51	0.0451	90.2
3	0.0456	19.71	0.0434	95.1
4	0.0555	23.26	0.0512	92.2
PART II†				
1	0.0998	44.94	0.0989	99.10
2	0.0918	40.39	0.0889	96.84
3	0.0902	41.04	0.0903	100.10
4	0.0974	44.26	0.0974	100.00
5	0.0967	44.68	0.0983	101.65
PART III‡				
1	0.1080	47.80	0.1052	97.41
2	0.0836	37.54	0.0826	98.80
3	0.0914	41.21	0.0907	99.23
4	0.0940	42.36	0.0932	99.15

\* The metaldehyde was depolymerized with  $H_2SO_4(1+4)$ , and a current of air of 5 bubbles per second was passed through the mixture held at 60°–70° C. for 1 hour. Recoveries were no better with much longer periods of time.

† Runs 1 and 2 were on the metaldehyde alone, while runs 3, 4, and 5 were on mixtures of metaldehyde (4%) with calcium arsenate (6%) and sawdust (90%). The procedure was the same as in Part I, except that steam distillation was included after the aeration. Determinations under No. 5 were made by A. Buell of the Food and Drug Administration.

‡ 1 N  $H_2SO_4$  used, the acid mixture aerated at 60°–70° C. for 1 hour, and 50 ml steam distilled from mixture. Runs 1 and 2 were recoveries on the metaldehyde alone. Runs 3 and 4 were made on mixtures of bran (97%) and metaldehyde (3%). This procedure is the one finally adopted.

TABLE 2.—*Blank determination on carriers*

MATERIAL	WEIGHT	CONDITIONS	0.1 N IODINE REACTING WITH "BOUND" DISULFITE
		A. $H_2SO_4(1+4)$	ml.
(1) Mixture of $As_2O_3$ and sawdust*	3.0	Aeration for 1 hr. at 60°–70° C. Steam distilled 50 ml.	1.38
(2) Sawdust	3.0	"	1.50
(3) Bran	3.0	"	1.71
		CONDITIONS	
		B. 1 N $H_2SO_4$	
(4) Sawdust plus $As_2O_3$ *	3.0	Aeration for 1 hr. at 60°–70° C. Steam distilled 50 ml.	0.14
(5) Sawdust	3.0	"	0.10
(6) Bran plus $As_2O_3$ *	3.0	"	0.05
(7) Bran	3.0	"	0.05

\* Approximately 0.5 gram of  $As_2O_3$  was used in each case.

from a change only in the concentration of the sulfuric acid from 1+4 to 1 N. The procedure finally adopted gave the blanks shown in Table 2 (B). Results of recovery experiments are shown in Table 1, Part III. The

average recovery on metaldehyde is 98.1 per cent and on metaldehyde in the presence of bran it is 99.2 per cent.

It may be concluded that the 1 *N* acid is strong enough to depolymerize the metaldehyde under the conditions of the method, but not strong enough to react appreciably with the bran or sawdust and to cause interference.

#### SUMMARY

A procedure for the determination of metaldehyde is described. It is based on the depolymerization to acetaldehyde and subsequent titration of the "bound" sulfite. Aeration to remove the acetaldehyde formed during depolymerization instead of after depolymerization is completed and steam distillation are factors that add greater precision to and shorten the procedure. The extent of interference of metaldehyde carriers was also studied.

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### COMPARISON OF THE OFFICIAL AND MACINTIRE-SHAW-HARDIN METHODS FOR DETERMINING AVAILABLE PHOSPHORIC ACID IN FERTILIZER MIXTURES CONTAINING PHOSPHATE ROCK SUPPLEMENTS\*

By L. F. RADER, JR., and WILLIAM H. ROSS (Fertilizer Research Division, Bureau of Plant Industry, Washington, D. C.)

At the 1939 meeting of this Association a report was given on the results obtained in a collaborative study of the relative merits of the official and MacIntire-Shaw-Hardin methods for determining the availability of phosphates (3). The standard samples submitted to the collaborators for use in the 1939 study were as follows:

1. Ammoniated superphosphate containing 8.66% of  $\text{NH}_3$ .
2. Tricalcium phosphate, precipitated.
3. Calcium hydroxyphosphate  $[3\text{Ca}_3(\text{PO}_4)_2] \cdot \text{Ca}(\text{OH})_2$ .
4. Calcined phosphate.
5. Tennessee brown-rock phosphate.

Table 1 gives the averages of the collaborative results reported in 1939 for total, available, and citrate-insoluble  $\text{P}_2\text{O}_5$  in these standard samples

\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 28-30, 1940.

TABLE 1.—Averages of results reported by collaborators in the analysis of standard phosphate samples by the official method and the MacIntire-Shaw-Hardin method for mixed fertilizers (per cent)

SAMPLE NO.	MATERIAL	TOTAL PHOSPHORIC ACID (P <sub>2</sub> O <sub>5</sub> )	AVAILABLE PHOSPHORIC ACID (P <sub>2</sub> O <sub>5</sub> )				CITRATE-INSOL. PHOSPHORIC ACID (P <sub>2</sub> O <sub>5</sub> )			
			OFFICIAL METHOD		MACINTIRE-SHAW-HARDIN METHOD		OFFICIAL METHOD		MACINTIRE-SHAW-HARDIN METHOD	
			1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM
1	Ammoniated Superphosphate	18.72	11.89	16.12	13.41	17.33	6.83	2.62	5.31	1.46
2	Tricalcium Phosphate	40.87	25.45	38.59	35.64	40.24	15.42	2.30	5.24	0.66
3	Calcium Hydroxyphosphate	42.03	12.03	19.26	19.90	28.86	30.02	22.83	21.42	13.10
4	Calcined Phosphate	37.22	33.57	34.31	33.27	35.34	3.68	2.95	3.96	1.89
5	Tennessee Brown Rock	33.59	2.93	3.75	4.03	6.58	30.64	29.86	29.98	27.08

when 1-gram and 0.5-gram samples were taken for analysis by both methods. The table shows that the MacIntire-Shaw-Hardin method (2) gave higher availability values for the  $P_2O_5$  in each of the samples with the exception of Sample 4 when a 1-gram sample was taken for analysis. In the case of the phosphate rock sample (Sample 5) the official method gave available  $P_2O_5$  values of 2.93 and 3.75 per cent when 1-gram and 0.5-gram samples, respectively, were taken for analysis. The corresponding values obtained with the MacIntire-Shaw-Hardin method as recommended for mixed fertilizers were 4.03 and 6.58 per cent.

The MacIntire-Shaw-Hardin method as applied to mixed fertilizers involves prior leaching with 100 ml. of citrated ammonium nitrate solution, steam digesting the residue in another 100 ml. of the solution, adjusting the *pH* of the leachate to 4.2 with nitric acid, mixing the two solutions, and making to volume. When the step of prior leaching with the citrated ammonium nitrate solution is omitted in the analysis of phosphate rock, as recommended by the authors of the method, availability ratings lower than those found with the official method are obtained (2). Thus a value of 2.47 per cent was found for the available  $P_2O_5$  in Sample 5 when prior leaching was omitted, as compared with the value of 2.93 per cent as found by the collaborators with the official method (Table 1).

When mixed fertilizers containing additive phosphate rock are analyzed by the MacIntire-Shaw-Hardin method the rock content of the sample is necessarily subjected to the step of preliminary leaching recommended for mixed fertilizers and not to the direct treatment recommended for phosphate rock as such. In the present paper a comparison is made of the official method and of the MacIntire-Shaw-Hardin method, as recommended for mixed fertilizers, for determining the available  $P_2O_5$  in ammoniated and non-ammoniated mixtures containing 10 per cent and 20 per cent of raw phosphate rock. The mechanical analysis of the phosphate rock used in the work was as follows:

	<i>per cent</i>
Through 100-mesh	100
Through 200-mesh	98
Through 300-mesh	92
Through 400-mesh	86

The formulas of the mixtures used in this comparative study are given in Table 2. Samples 1, 2, 3, and 4 were non-ammoniated mixtures, and Samples 5 and 6 were ammoniated mixtures. Sample 1 contained 10 per cent of dolomite and no phosphate rock, whereas Samples 2 and 5 contained 10 per cent of phosphate rock but no dolomite. Samples 4 and 6 contained dolomite and 10 per cent of phosphate rock. Sample 3 contained 20 per cent of phosphate but no dolomite. The results obtained by L. F. Rader, Jr. of the Bureau of Plant Industry and by L. J. Hardin of the

TABLE 2.—*Formulas of a 4-8-4 mixed fertilizer*

MATERIAL	POUNDS PER TON OF MIXED FERTILIZER					
	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6
Superphosphate, $P_2O_5 = 19.4\%$	825	825	825	825	—	—
Ammoniated Superphosphate, $P_2O_5 = 18.9\%$ ; $N = 2.06\%$	—	—	—	—	846	846
Ammonium Sulfate, $N = 20.6\%$	291	291	291	291	209	209
Sodium Nitrate, $N = 16.4\%$	183	183	183	183	183	183
Uramon, $N = 42.0\%$	71	71	71	71	71	71
Potassium Chloride, $K_2O = 51.8\%$	154	154	154	154	—	—
Manure Salts, $K_2O = 30.5\%$	—	—	—	—	262	262
Phosphate Rock (98% through 200 mesh)	—	200	400	200	200	200
Dolomite	200	—	—	276	—	229
Filler (quartz flour)	276	276	76	—	229	—
	2000	2000	2000	2000	2000	2000

Tennessee Agricultural Experiment Station in the analysis of these samples are given in Table 3.

It has been contended that the addition of phosphate rock to mixtures

TABLE 3.—*Available  $P_2O_5$  in 4-8-4 fertilizer mixtures with and without phosphate rock supplement as determined by official and MacIntire-Shaw-Hardin methods (per cent)*

SAMPLE NO.	ANALYST	OFFICIAL METHOD			MACINTIRE-SHAW-HARDIN METHOD		
		PHOSPHORIC ACID ( $P_2O_5$ )			PHOSPHORIC ACID ( $P_2O_5$ )		VARIATION FROM OFFICIAL METHOD
		TOTAL	INSOLUBLE	AVAILABLE	INSOLUBLE	AVAILABLE	
1	Hardin	8.60	0.17	8.43	0.20	8.40	-0.03
	Rader	8.44	0.21	8.23	0.21	8.23	0.00
2	Hardin	11.80	3.33	8.47	3.10	8.70	+0.23
	Rader	11.67	3.27	8.40	3.19	8.48	+0.08
3	Hardin	15.30	6.38	8.92	6.10	9.20	+0.28
	Rader	15.13	6.50	8.63	5.85	9.28	+0.65
4	Hardin	11.80	3.45	8.35	3.20	8.60	+0.25
	Rader	11.63	3.49	8.14	3.26	8.37	+0.23
5	Hardin	11.90	3.47	8.43	3.30	8.60	+0.17
	Rader	11.63	3.60	8.03	3.13	8.50	+0.43
6	Hardin	11.90	3.60	8.30	3.55	8.35	+0.05
	Rader	11.76	3.67	8.09	3.51	8.25	+0.16

that are prepared and stored under certain conditions induces a reaction between the rock and the acidic components of the mixture and a slight resultant increase in the  $P_2O_5$  availability in the mixture (4). The fertilizer

TABLE 4.—*Available  $P_2O_5$  in commercial fertilizers as determined by the official and MacIntire-Shaw-Hardin methods (per cent)*

(H. B. Siems, Analyst)

SAMPLE NO.	GRADE	AGE OF FERTILIZER	OFFICIAL METHOD	MACINTIRE-SHAW-HARDIN METHOD	
			AVAILABLE $P_2O_5$	AVAILABLE $P_2O_5$	VARIATION FROM OFFICIAL METHOD
		weeks			per cent
<i>Commercial Superphosphates</i>					
819	—	9	19.05	18.98	-0.07
824	—	9	18.84	18.70	-0.14
825	—	9	19.30	19.52	+0.22
826	—	9	19.12	19.10	-0.02
847	—	10	19.14	19.25	+0.11
<i>Commercial Ammoniated Mixtures Made Non-acid Forming with Dolomite</i>					
800	3-13-0	47	11.16	12.44	+1.28
801	4-12-4	8	11.59	12.93	+1.34
802	4- 8-0	30	8.02	8.45	+0.43
803	4-12-0	30	11.62	12.15	+0.53
804	6-12-6	21	11.60	11.75	+0.15
805	4- 8-0	30	8.02	8.45	+0.43
813	5-13-8	—	12.73	12.68	-0.05
817	4-12-4	—	12.23	12.60	+0.37
871	3-13-2	56	11.53	11.55	+0.02
872	6-10-0	47	8.03	8.28	+0.25
873	4-10-4	38	9.80	10.43	+0.63
924	3-14-0	13	14.80	14.78	-0.02
925	2-16-0	30	15.83	15.48	-0.35
957	4-12-4	95	11.28	11.75	+0.47
958	3-13-0	8	12.94	13.61	+0.67
959	4-12-4	21	11.93	12.16	+0.23
960	4-12-4	52	11.62	12.18	+0.56
961	4-10-7	52	9.77	10.29	+0.52

mixtures used in the present work were prepared from dry materials, however, and the mixtures were analyzed before any appreciable decomposition of the phosphate rock in the mixtures could take place. The data in Table 3 show that under these conditions the addition of 10 per cent of phosphate rock to the fertilizer mixtures, whether ammoniated or non-ammoniated, has little or no effect on the availability of the  $P_2O_5$  in the mixture as determined by either the official method or the MacIntire-Shaw-Hardin procedure. The latter method, however, gave on an average slightly higher values for the available  $P_2O_5$  in the samples. When the rock in the mixture was increased to 20 per cent, a small increase in available



$P_2O_5$  was indicated by the official method and a still greater increase by the MacIntire-Shaw-Hardin method. The presence or absence of dolomite in the mixtures under the conditions in which the mixtures were prepared and analyzed did not seem to have any appreciable effect on the results as determined by either method.

In Table 4 are given results obtained by H. B. Siems, Swift and Co. Fertilizer Works, in the determination of available  $P_2O_5$  in cured commercial superphosphate samples and in commercial ammoniated mixed fertilizers that had been made non-acid forming by the addition of dolomite. All samples were analyzed by both the official and MacIntire-Shaw-Hardin methods. The table shows that both methods gave closely agreeing results for available  $P_2O_5$  in the cured superphosphate samples, but that slightly higher results on an average were obtained with the MacIntire-Shaw-Hardin method in the analysis of the commercial mixtures. The results submitted by Siems are thus in agreement with those given in Table 3.

It may be concluded from this work and from that described in previous publications that (a) the official and MacIntire-Shaw-Hardin methods give closely agreeing results in the determination of available  $P_2O_5$  in commercial superphosphates of the ordinary type; (b) the MacIntire-Shaw-Hardin method gives higher results than does the official method for available  $P_2O_5$  in basic phosphates such as tricalcium phosphate, heavily ammoniated superphosphates (1), and reverted phosphates (2); (c) the MacIntire-Shaw-Hardin method as recommended for mixed fertilizers gives higher results for available  $P_2O_5$  than does the official method when applied to the analysis of phosphate rock as such (1, 3), but the reverse is true when the MacIntire-Shaw-Hardin method is used as recommended for phosphate rock (2); and (d) that the MacIntire-Shaw-Hardin method gives slightly higher results for available  $P_2O_5$  in ammoniated and non-ammoniated mixtures of the ordinary type.

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## SAMPLING OF FERTILIZERS

By WILLIAM H. ROSS, JOHN O. HARDESTY, and L. F. RADER, JR.  
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The physical properties of the fertilizer mixtures that are now on the market differ considerably. A mixture that is finely ground, or one in which all the components are of the same particle size, will not segregate, whereas a mixture that is made up of components that differ markedly in particle size will segregate readily. Thus it may happen that a fertilizer mixture that is actually up to guarantee as a whole may, nevertheless, be reported as falling below guarantee in one or more of the plant food elements due to failure of the inspector to obtain a representative sample, or to segregation of the mixture when the sample is being prepared for analysis.

During the past year a collaborative study was made of the fineness to which mixed fertilizer samples should be ground for analysis in order to eliminate the effect of segregation. A report on the results obtained by the collaborators has already been presented at this meeting of the Association by Ross, Rader, and Hardesty (see p. 253). This report shows clearly that notwithstanding the care that may be taken in the collection of fertilizer samples erratic results may be obtained when samples are only fine enough to pass a 1-mm. sieve, as directed in the official method. A recommendation was accordingly made that dry mixed fertilizers that tend to segregate be ground to pass a 35-mesh sieve. This procedure is now common practice in many laboratories.

The official method for collecting fertilizer samples for analyses, *Methods of Analysis*, A.O.A.C., 1940, 20, is worded as follows:

Each official sample shall consist of at least 1 lb. of material taken in following manner: Use sampler that removes a core from top to bottom of bag. Take cores from not less than 10% of bags present unless this process necessitates cores from more than 20 bags, in which case take core from 1 bag for each additional ton represented. If less than 100 bags, sample not less than 10 bags; if less than 10 bags, sample all bags. Thoroly mix portions taken on clean oilcloth or paper, reduce by quartering to quantity of sample required, and place in air-tight container.

This procedure for sampling fertilizers was adopted at the 35th meeting of this Association in 1919 (5). The average mixed fertilizer in use at that time contained about 60 per cent of superphosphate and had a total plant food content of approximately 15 per cent. The superphosphate used in the preparation of these mixtures was for the most part a finely divided material, and a considerable proportion of most of the mixed fertilizers that were then on the market was therefore also finely divided. Data published by the Fertilizer Division of the Department of Agriculture as recently as 1935 show that about 50 per cent of the low-analysis mixtures then on

the market was made up of material that passed a 40-mesh screen (12). A mixture of this kind, which contains a sufficient proportion of finely divided material to more than fill the interstices between the larger particles, exhibits little tendency to segregate.

Within recent years, however, there has been a tendency to increase the particle size of superphosphate and other fertilizer materials with a view to improving their drillability. The particle size of the components of a fertilizer mixture may be increased without causing serious segregation provided the sizes of the particles of the different components are approximately the same. Most fertilizer mixtures are not made up of materials of the same particle size, and in many mixtures, particularly high-analysis mixtures, the proportion of finely divided materials present may be insufficient to fill the interstices between the larger particles. Mixtures of this kind are therefore subject to segregation when handled or agitated.

A survey made by Lodge (8) in 1917 shows that the methods then in use for sampling fertilizers varied greatly in different parts of the country. Other data on the effect of improper sampling on fertilizer analyses have also been published from time to time (1, 4, 7, 8, 13). Inasmuch as the necessity for uniform sampling would now seem to be more pressing than formerly, a questionnaire on sampling was addressed to the state control officials in the states in which the fertilizer consumption exceeds 10,000 tons per annum.

The questionnaire requested information on (1) the type of sampler used in sampling fertilizers; (2) the number of bags sampled; (3) the procedure followed in reducing the sample to the size sent to the analytical laboratory; (4) the weight of the sample sent to the laboratory; (5) the method of preparing the sample for analysis; (6) the fineness to which the sample is ground for analysis; and (7) as to whether the sampling method used is prescribed in the fertilizer control law of the state, or whether this matter is within the discretion and regulation of the control official.

Replies were received from officials in 32 states, or all to whom the questionnaire was sent, excepting those in the states of Georgia, Arkansas, and Illinois.

The procedure now used in all of these 32 states for sampling fertilizers consists in taking a core of the fertilizer from the top to the bottom of the bag. The samplers used in the different states for taking this core of fertilizer are of three general types. The simplest of these types consists of a slotted, single, brass tube. Samplers of this type may be grouped into three sub-types: (a) the open-end, half-curved butter tryer; (b) the open-end, slotted tube, such as the Kellogg sampler (6); and (c) the solid-end, slotted tube. These slotted tube samplers are sometimes provided with a raised cutting edge on one side of the slot. The most approved method of operating the slotted, single-tube sampler is to insert it into the bag with the slot down and to have the bag in a horizontal position. After the tube

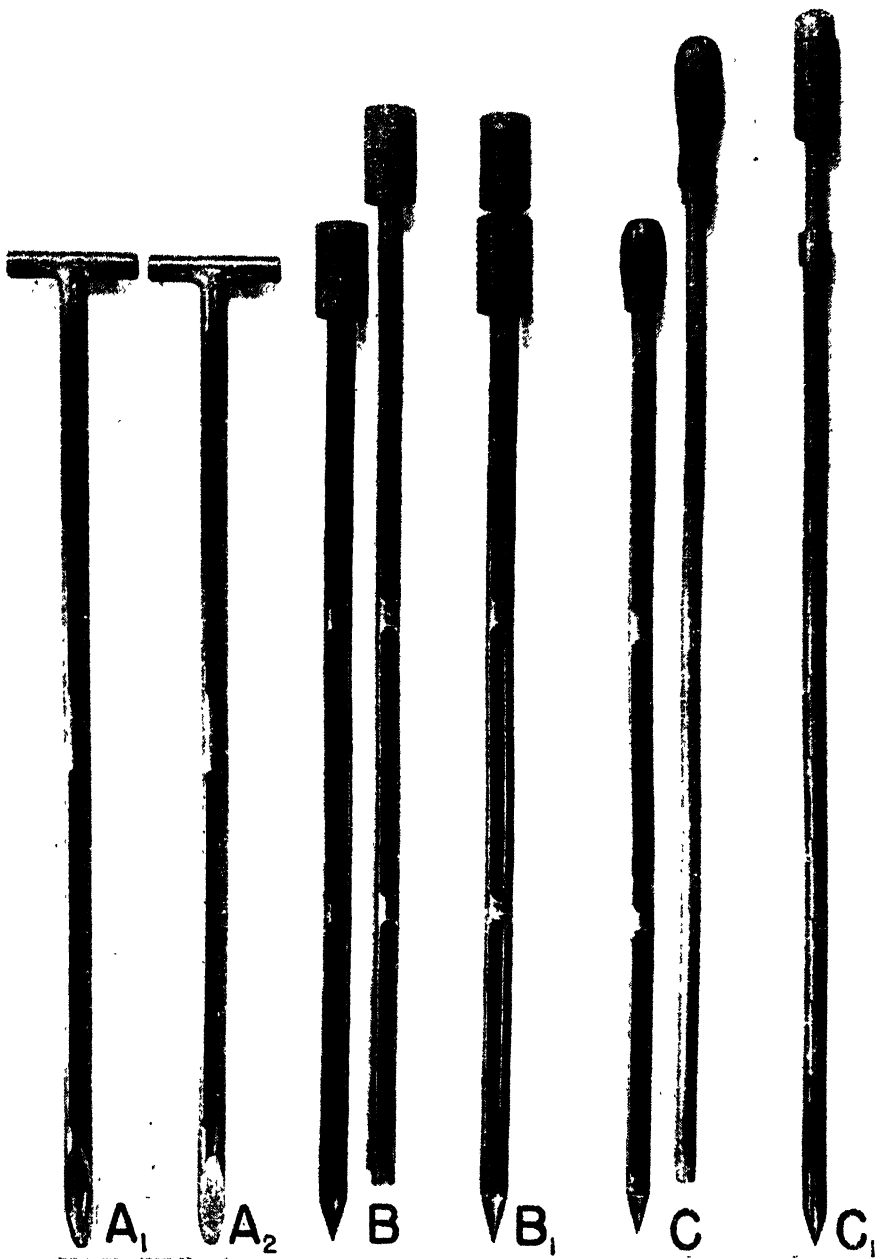


FIG. 1.—FERTILIZER SAMPLERS

- A<sub>1</sub>.—Slotted, single-tube, open-end sampler.
- A<sub>2</sub>.—Slotted, single-tube, closed-end sampler.
- B. —Slotted, double-tube, solid pointed-end sampler, disassembled.
- B<sub>1</sub>.—Slotted, double-tube, solid pointed-end sampler, assembled and partly open.
- C. —Tube and rod, solid pointed-end sampler, disassembled.
- C<sub>1</sub>.—Tube and rod, solid pointed-end sampler, assembled and partly open.

has been inserted it is turned over so that the slot is on top, and the bag is shaken to fill the tube when the sampler is not provided with a cutting edge; samplers that have a cutting edge are preferably turned over several times after being inserted into the fertilizer, and shaking of the bag is then unnecessary.

The second type of sampler differs from the first type in that it is provided with a brass rod, which is placed within the brass tube when the sampler is to be inserted into the fertilizer. When a sample is to be taken the rod is withdrawn from the tube to permit the flow of fertilizer into the tube.

The third type of sampler consists of two telescoping, slotted, brass tubes. In the Indiana sampler (10) the outer tube terminates in a solid pointed end, which permits the sampler when closed to be inserted the full length of a bag before any material can enter the sampler chamber. The sampler is then opened by turning the inner tube until the slots in both tubes are in alignment, following which operation the bag is agitated sufficiently to cause the fertilizer to flow into the chamber. The chamber is again closed before the sampler is withdrawn.

The slotted, single-tube samplers are used in more states than are any other type, and of the different makes of samplers, the Indiana instrument is the most widely used. It is theoretically more accurate than any other make of sampler, and this is also claimed to be true of its practical application by many of its users (1, 7, 9). However it is sometimes more difficult to operate double-tube and tube-and-rod samplers than the single-tube samplers because they frequently become clogged (2). The solid-end, single-tube sampler with raised cutting edge would seem to be sufficiently accurate to meet requirements, and it has the advantage that it eliminates the mechanical difficulties sometimes encountered with the double-tube, and tube-and-rod samplers. As was shown by Lodge (8), open-end, single-tube samplers can not be depended upon to collect representative samples of fertilizer mixtures.

The replies received from the state control officials show that the double-tube sampler is used in 11 states, the tube-and-rod samplers in 2 states, and the single-tube samplers in 18 states.

In 24 of the 32 states reporting cores are taken from all bags, as directed in the official method, if the lot to be sampled is less than 10. In 6 states the law specifies that not less than 5 out of the 10 bags be sampled, in one state 2 to 5 bags in 10, and in another state no definite number is specified. When the lot to be sampled exceeds 10, a core is taken from 20 per cent of the bags in 4 states, from 10 per cent of the bags in 21 states, from 5 per cent of the bags in 6 states, and from no set proportion in one state.

One core is taken parallel to the sides and from the top to the bottom of each bag sampled in 27 states and two or three cores are thus taken in 1

state. In the states of Ohio, Indiana, Florida, and California from one to three cores are taken in a diagonal direction from the top to the bottom of the bag.

In 9 states the entire sample collected by the inspector is sent to the laboratory, while in the remaining 23 states a portion only of the original sample is selected for the laboratory by quartering. The sample sent to the laboratory amounts to less than half a pound in 2 states, to about 1 pound in 18 states, and to 2 pounds or more in the remaining 12 states.

The entire sample submitted to the chemist in 18 states is ground to pass a 2 mm. or a 10-mesh sieve before being subdivided for analysis. In 5 states the sample is ground to pass a 20-mesh sieve and in 9 states the sample is not screened before it is subdivided.

The sample selected for analysis amounts to less than a fourth of a pound in 8 states, from one-fourth to one-half a pound in 19 states, and from one-half to one pound in 5 states. In 18 states this sample is ground before analysis to pass a 1 mm. sieve with circular openings as directed in the official method; in 4 states it is ground to pass a 20-mesh sieve; and in the remaining 10 states it is ground to pass a 0.5 mm. sieve with circular openings or a wire-mesh sieve that varies in different states from 20- to 100-mesh.

The fertilizer control laws in 17 states specify that the A.O.A.C. method of sampling or a method similar to that of the A.O.A.C. be used; those in 14 other states have no specific requirements as to methods of sampling; and in only one state does the law specify a method of sampling that is different from the official method. The survey also shows that the methods used in sampling fertilizers in the different states are now much more uniform than they were formerly (8). Most of these methods should provide fertilizer samples that are representative of an entire shipment unless serious segregation has taken place in the bag or the fertilizer was inadequately mixed before it was placed in the bag. This question is discussed in a letter received from W. C. Geagley, Chief Chemist, Michigan Department of Agriculture. Mr. Geagley's letter reads, in part, as follows:

For some reason fertilizer control work presents many and varied problems, which seem to be more complicated in some sections than in others, and apparently no logical explanation can be given for what appears to be erratic results of analysis on complete fertilizers that have been sampled by inspectors.

With our force we have endeavored to simplify the procedure as much as possible and to maintain a uniform system so that different inspectors could closely follow this plan. Many times six inspectors will sample the same firm's fertilizer, and results in the laboratory will show close to guarantee in the six cases and the samples collected by a seventh inspector from a shipment of the same grade of fertilizer produced by the same firm will show widely divergent results.

We have just about come to the conclusion that in many instances the difficulty is in the small batch mixing by fertilizer companies rather than in segregation of the fertilizer in the bag during shipment. Instead of preparing different grades of

fertilizers in large quantities, each load of fertilizer is mixed separately, and this with careless weighing and mixing probably accounts for more variations in analysis than any other single factor.

The following statement bearing on the same subject was received from J. D. Patterson, Chief Chemist, Oregon State Department of Agriculture:

We have not noticed any difficulty in sampling during the past year. Previous years we have encountered very poorly mixed materials in the field, which made sampling difficult. I believe this has been taken care of by the manufacturers.

If fertilizer bags have been shipped standing up, any of the samplers having a solid pointed end should give a representative sample even though segregation has taken place in the mixture. However, if the bags are shipped in a horizontal position, a core taken from one end of the bag to the other parallel to the sides of the bag might not give a representative sample if the mixture had segregated in shipment. The procedure followed in several of the states of taking a sample diagonally through the bag would therefore seem to be a good one, and one that might very well be adopted by all control officials. One sample collected in this way might be more representative than would several samples taken parallel to the sides of a bag or of several bags. This feature of sampling fertilizers is discussed in a letter received from Dr. Alvin J. Cox, Chief, Bureau of Chemistry, California Department of Agriculture:

One of the items of major importance not mentioned in your letter is the extreme difficulty in mixing materials of varying specific gravity, as for example fish meal and sulfate of potash. In addition to the filled-out questionnaire, which we are enclosing, we should like to make a few comments pertaining thereto.

In drawing a sample of commercial fertilizer the tryer with the slot down is inserted at one corner of the bag, preferably the top or ear end, and thrust diagonally towards the opposite corner of the sack. The tryer is then turned so that the slot is up, withdrawn, and emptied into a bucket. In windy weather the tryer is emptied through a 1½ inch tube shield, and the bucket is covered between tryer samples. As a point of interest we use the Ace Fastener Corp. Model 702 clipper to seal tryer holes in paper bags and the tryer holes in burlap bags of free-running materials.

When a sample analyzes below guarantee, another subsample of the original is ground and analyzed. If these two subsamples of the original do not agree, the whole of the remaining original sample is ground to pass through a 1-mm. sieve.

Other features not mentioned in the official method, but which contribute towards accuracy of sampling, have been adopted in various control laboratories. Thus, in Vermont a specially designed flat-bottomed scoop is used to transfer the sample, after being mixed, to its container, and in Mississippi the mixing of fertilizer samples is facilitated by use of two metal plates.

The report that has already been given at this meeting of the Association on Preparation of Fertilizer Samples for Analysis (see p. 253) shows conclusively that marked segregation may take place in mixtures that have been ground for analysis. It would seem, therefore, that still greater segre-

gation might take place in the unground mixtures when agitated, as in the process of their distribution in the field. That this actually takes place has been demonstrated by Mehring and his associates (12) in field tests with the fertilizer attachments on a grain drill.

For many years numerous field tests have been made to determine the best fertilizer grades for different soils and crops, and the number of grades now sold in this country is probably in excess of 1000 (11). If a fertilizer of a certain grade is better adapted to a given set of conditions than any other, then the effectiveness of the fertilizer must necessarily be impaired by any segregation that would change the grade of the fertilizer in the process of its distribution in the field. While it is the duty of the control official to secure representative samples for analysis, there would also seem to be an obligation on the part of the manufacturer to place on the market non-segregating mixtures of uniform composition.

The Fertilizer Research Division of the Bureau of Plant Industry is now making a further study of the factors involved in the segregation of fertilizers and of methods for preventing it. A report on this phase of the work will be presented at a later date.

#### RESULTS FROM QUESTIONNAIRE ON SAMPLING OF FERTILIZERS

<i>Method of Collecting Samples</i>	<i>No. of States</i>
1. Type of sampler used in sampling bags:	
A. Slotted single tube . . . . .	18
B. Slotted double tube . . . . .	11
C. Slotted tube and rod . . . . .	2
2. Number of bags sampled when:	
A. More than ten bags are present:	
(a) Cores from 20% . . . . .	4
(b) Cores from 10% . . . . .	21
(c) Cores from 5% . . . . .	6
(d) Cores from no definite number . . . . .	1
B. Less than ten bags are present:	
(a) Cores from all . . . . .	24
(b) Cores from not less than 5 . . . . .	6
(c) Cores from 2 to 5 . . . . .	1
(d) Cores from no definite number . . . . .	1
3. Method used in taking sample:	
A. One core from each bag taken parallel to the sides . . . . .	27
B. Two or three cores from each bag taken parallel to the sides . . . . .	1
C. Cores taken diagonally . . . . .	4
4. Sample sent to laboratory:	
A. The entire sample . . . . .	9
B. A portion of the sample . . . . .	23
5. Weight of sample sent to laboratory:	
A. Less than 1 lb. . . . .	2
B. 1 lb. or more . . . . .	18
C. 2 lbs. or more . . . . .	12



*Method of Preparing Sample for Analysis*

1. Mesh of screen through which the Inspector's sample is passed before it is subdivided for laboratory sample:
  - A. 2 mm. or 10 mesh. . . . . 18
  - B. 20 mesh. . . . . 5
  - C. Not screened. . . . . 9
2. Weight of sample prepared for laboratory:
  - A. Less than  $\frac{1}{2}$  lb. . . . . 8
  - B.  $\frac{1}{2}$  to  $\frac{3}{4}$  lb. . . . . 19
  - C.  $\frac{3}{4}$  to 1 lb. . . . . 5
3. Size of screen through which ground sample is passed:
  - A. 1 mm. circular opening. . . . . 18
  - B. 20 mesh. . . . . 4
  - C. 0.5 mm. circular opening, or finer than 20 mesh. . . . . 10

*General*

1. Fertilizer control law of state specifies the use of:
  - A. The A.O.A.C. method of sampling or one similar to that of A.O.A.C. 17
  - B. A method different from that of the A.O.A.C. . . . . 1
  - C. No definite method of sampling. . . . . 14

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DETERMINATION AND SIGNIFICANCE OF PHENOLS  
IN VANILLA EXTRACT\*

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The estimation of vanillin has sometimes been used as an indication of the quality of both vanilla beans and vanilla extracts. Attempts have also been made to follow the chemistry of the curing process (during which vanillin is liberated) and to get an objective measure of its success or failure in a given case, by the determination of vanillin. The significance of such data may be questioned on the ground that beans from Mexico and from Tahiti are both comparatively low in vanillin, but the Mexican beans are most highly regarded and the Tahitian beans are usually regarded as inferior to other sorts in quality. The vanillin content is evidently not a criterion of the flavoring value, and it follows that other substances in the natural product contribute largely to the desired flavor.

In this Laboratory an attempt was made to estimate the total phenol present throughout the curing process. The results of an admittedly small experiment show an approximate but interesting correlation between the quality of vanilla extracts and the content of phenol. They indicate, furthermore, that phenols other than vanillin are responsible for much of the flavoring value of the extract. The data are given and discussed at the conclusion of this paper. The main purpose at present is to record the method by which the determinations were made. The total phenol so determined was calculated as per cent of vanillin on the dry weight of the beans, and is referred to as the "phenol value."

## METHOD

The method utilizes the simple colorimetric methods for phenols that have been developed in recent years. By means of a photometer the blue color developed in the phenol reagent of Folin and Ciocalteau (1) is measured. The principle of the determination is the same as that of the official colorimetric method for vanillin given by the A.O.A.C. (2). Another photometric method for vanillin, in which a different reagent (*o*-iodoxy ammonium benzoate) is used, has been described by Daniels, Emery, and Prather (3). The method presented here is patterned after these methods, but it aims to include other phenolic substances, of which some appear to possess desirable flavoring properties.

The blue color developed in the phenol reagent by the addition of vanillin was first investigated. A Coleman double monochromator spectro-

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\* Enzyme Research Laboratory Contribution No. 64.

photometer was used. It consists of a device for comparing the transmission of light of a suitable wave length through the colored solution with its transmission through water or a solution containing only the blank reagents. In this case water was used. The quantities of the reagents used approximated those recommended by Anson (4) for the estimation of tyrosine. Thus a standard aqueous solution of pure vanillin, previously recrystallized from water, was prepared. A known quantity of this solution was diluted to 5.0 ml. To this was added 10.0 ml. of 0.4 *N* sodium hydroxide, followed by 3 ml. of the diluted phenol reagent of Folin and Ciocalteu corresponding to 1.0 ml. of the reagent prepared according to the reference

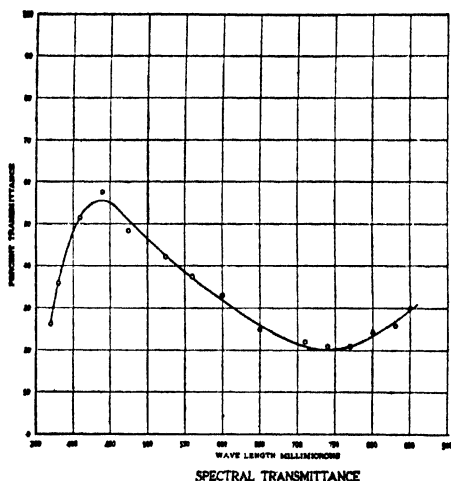


FIG. 1.—SPECTRAL TRANSMITTANCE.

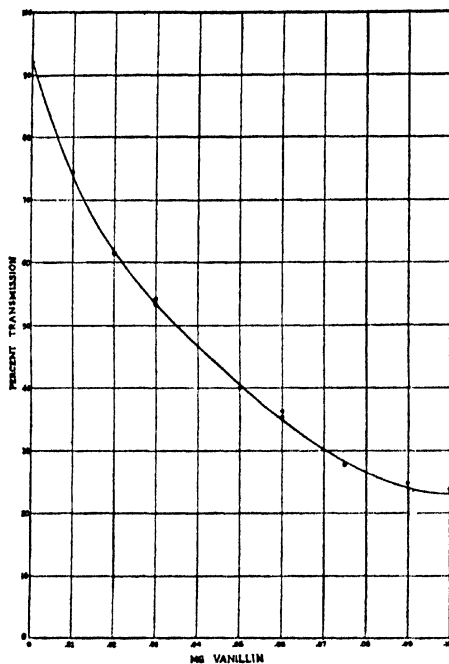


FIG. 2.—CALIBRATION CURVE WITH VANILLIN.

given (1). The volume of the reacting mixture is therefore 18.0 ml. Because the photometer measures concentration, the values given here refer to the total vanillin present in this volume, and therefore present in the 5.0 ml. of solution used before the addition of the reagents.

The transmission of the blue solution produced in the reaction between vanillin and the reagent was observed to show a broad minimum between 725  $m\mu$  and 770  $m\mu$ ; thus the color measurement was most delicate in this range (Figure 1). Subsequent measurements were made at 750  $m\mu$ .

The intensity of color was found to decrease with the time the solution stood. This variation is not great, as indicated by Table 1, but it is enough to require that readings be made at a specified time after the reagent is added. An interval of 5 minutes was chosen in this work.

A standard curve was next prepared from the pure vanillin. The per cent transmission, plotted against the vanillin present, is shown in Figure 2. If the quantity of vanillin is plotted against the log of per cent transmission the curve obtained is not a straight line. Therefore there appears to be no advantage in using the customary semilogarithmic plot in this particular case.

TABLE 1.—*Showing the instability of the vanillin color*

TIME	PER CENT TRANSMISSION AT 740 M $\mu$	
minutes	1	2
0	—	
4	19.3	
7	19.5	
10	20.5	21.0
20		23.5
25		25.3
30		27.3

Since vanilla extract contains alcohol, the influence of this constituent upon the reaction was studied. Suitable quantities of alcohol were added to pure vanillin, which was then determined by reference of the color produced to the curve in Figure 2. No appreciable effect was observed from quantities of alcohol comparable to several times what might be present in vanilla extracts. Table 2 shows the results of the experiment.

TABLE 2.—*Effect of alcohol*  
(Vanillin standard diluted with water and alcohol.  
Amounts in single determinations)

VANILLIN ADDED	ALCOHOL	VANILLIN FOUND
mg.	mg.	mg.
0.0400	0	0.0415
.0400	32	.0410
.0400	16	.0426
.0400	8	.0422

The method outlined here was finally tested by the addition of known quantities of vanillin to a typical vanilla extract. The added vanillin was measurable with satisfactory accuracy, as shown in Table 3.

*Detailed Procedure.*—0.50 ml. of vanilla extract was diluted to 500 ml. with water. Of this dilution 5.00 ml. was placed in a 50 ml. Erlenmeyer flask; 10.0 ml. of 0.4 N sodium hydroxide was then added, followed by 3.0 ml. of the diluted phenol reagent (1). (The quantity of alkali should be only slightly more than that required to neutralize the acid of the reagent.) The flask was agitated during the addition of the reagent, which occurred, as is customary, in a rapid succession of discrete drops. After the solution had stood a total of 5 minutes, the per cent transmission with respect to water was read and translated on the standard curve to vanillin. It is important, with the instrument specified here, to use the same tubes for water and colored solution that were used in making the standard curve. Since this requires only a few minutes, the replacement of an old tube is not a serious matter.

## RESULTS

This method for "total phenols" was recently applied to over 40 vanilla extracts prepared\* in the Puerto Rico Experiment Station at Mayaguez from beans of known origin, including many Puerto Rican beans cured by

TABLE 3.—*Recovery of added vanillin from vanilla extract\**

VANILLIN ADDED	VANILLIN FOUND	ADDED VANILLIN RECOVERED
mg.	mg.	mg.
None	0.0265	
None	.0265	
None	.0500†	
	Av. 0.0256	
0.0100	0.0353	0.0097
.0200	.0468	.0212
.0300	.0567	.0311
.0500	.0717	.0461

\* 0.50 ml. of vanilla extract, diluted to 500 ml. with water and additional vanillin solution. Single determinations on 5.0 ml.

† 1.00 ml. of vanilla extract used as above.

several modifications of the Bourbon process. Table 4 shows the data obtained with extracts whose content of vanillin was also determined by a gravimetric method (2). The results for total phenol and phenol not extracted by ether are expressed as per cent of vanillin of the dry weight of the beans used to make the extract.

## DISCUSSION

The method here described for determining a phenol value was developed primarily as an aid in following some of the chemical changes that occur during the process of curing vanilla beans. Studies to this end are in progress.

When the actual vanillin content is also known, the difference between this and the total phenol is a measure of phenolic substances other than vanillin present in the extract. The amount of such other phenols may be some indication of the quality of the vanilla, as appears from the data in Table 4 on beans of foreign origin. These beans may be graded by the price in descending order as follows: Mexico beans, Madagascar beans, Comores beans, and Tahiti beans. It may be seen that of the four sorts analyzed, the higher the grade is considered to be, the greater the quantity of phenols other than vanillin. The same may be said of the ether-insoluble phenols.

The data in Table 4 also indicate differences between samples of Puerto Rican beans. It was impractical to grade these beans, however, because the

\* Standard strength vanilla extracts were prepared, e.g. 10 grams of beans for every 100 ml. of extract; 50% alcohol by volume was used for extraction; and compensation was made in all cases for the moisture content of the beans. The beans were cut in a Wiley Mill to pass a 2 mm -mesh sieve and macerated for 30 days in 60% of the total solvent. After percolation of this extract, the bean residues were washed with the reserved solvent and with additional 50% alcohol until the required volume was completed.

differences were less striking than those between the foreign samples, and were apparently of such a nature as to lead grading experts to disagree among themselves. Only small quantities of the beans were at hand, so it was impossible to grade them by their price on an open market.

TABLE 4.—Data on extracts from vanilla beans of known origin

PUERTO RICAN BEANS							
RUN	MATURITY	"KILLING" TREATMENT <sup>1</sup>	MOISTURE	PHENOL VALUE	VANILLIN, GRAVIMETRIC METHOD <sup>2</sup>	PHENOL NOT EXTRACTED WITH ETHER <sup>3</sup>	RATIO, VANILLIN/PHENOL VALUE
V-1	Green	Ethylene 16 hrs. <sup>4</sup>	per cent 25.92	per cent 6.90	per cent 4.05	per cent 1.43	0.59
V-2	Green	Ethylene 16 hrs. Frozen 20 hrs.	27.11	6.70	3.57	1.28	0.53
V-3	Green	Frozen 20 hrs.	27.59	7.30	4.39	1.30	0.60
V-4	Green	Rubbed with alcohol, frozen 20 hrs.	27.74	6.90	3.54	1.25	0.51
T-1	Green	Dipped in hot <sup>5</sup> water	27.50	7.30	4.07	1.30	0.56
T-2	Green	Frozen 4 hrs.	26.56	9.00	4.31	1.80	0.48
T-3	Green	Dipped in hot water, frozen 4 hrs.	26.46	7.00	3.79	1.10	0.54
T-4	Green	Frozen 4 hrs., dipped in hot water	26.78	7.40	3.80	1.30	0.51
T-5	Yellow <sup>6</sup>	Dipped in hot water	27.22	7.10	3.50	1.10	0.49
T-6	Yellow <sup>6</sup>	Frozen 4 hrs.	27.87	8.40	3.87	1.46	0.46
T-7	Yellow <sup>6</sup>	Dipped in hot water, frozen 4 hrs.	28.45	6.20	3.52	.92	0.57
T-8	Yellow <sup>6</sup>	Frozen 4 hrs., dipped in hot water	26.05	6.10	3.56	1.04	0.58

## FOREIGN BEANS

SOURCE	MOISTURE	PHENOL VALUE	VANILLIN <sup>2</sup>	PHENOL VALUE MINUS VANILLIN	PHENOLS NOT EXTRACTED WITH ETHER <sup>3</sup>	RATIO, VANILLIN/PHENOL VALUE
	per cent	per cent	per cent	per cent	per cent	
Mexico	37.69	5.13	1.94	3.19	0.71	0.38
Madagascar	32.32	6.50	3.75	2.75	0.63	0.58
Comores	31.91	5.00	2.83	2.17	0.59	0.57
Tahiti	30.41	3.24	1.84	1.40	0.38	0.57

<sup>1</sup> After being killed, the beans were sweated in electric ovens at 50° C. until proper flexibility was obtained, then dried on racks at room temperature to approximately 65% loss in weight and finally conditioned in wooden trunks for 6 months.

<sup>2</sup> Gravimetric method of A O A C. (2).

<sup>3</sup> Residual phenol after first series of four ether extractions in gravimetric method was determined colorimetrically by procedure outlined in this paper.

<sup>4</sup> Concentration of ethylene used was 1 part in 10,000 parts of air.

<sup>5</sup> Beans were immersed in water at 80° C. for 3 periods each of 10 seconds at 30 second intervals.

<sup>6</sup> Yellow refers to beans with yellow blossom ends.

It is clear that the vanillin content can not be universally accepted as a criterion of quality. The Mexican beans of admittedly high quality were found to contain the same amount of vanillin as did Tahitian beans of admittedly low grade. In most cases, however, the vanillin extracted by alcohol under the conditions used bears a remarkably constant relation to the total phenol similarly extracted. Table 4 shows that the vanillin usually runs between 50 and 60 per cent of the total phenols. In the majority of cases, therefore, the phenol value is a satisfactory indication of the vanillin content. The prominent exception to this statement shown by the Mexican sample is due to its relatively low vanillin content, which may indicate a loss of vanillin by evaporation during the curing process, not surprising in view of the fact that Mexican beans are sun cured. A slightly lower ratio of vanillin to total phenol is evident in some samples of Puerto Rican beans that were frozen as an initial step in the curing process (notably Experiments T-2 and T-6). In these cases, however, the vanillin content is abnormally high; the ratio is low because the quantity of phenols other than vanillin is even higher. The method of curing is thus undoubtedly reflected in the analytical data of Table 4.

It can be concluded from these results that phenolic bodies other than vanillin contribute to the flavor and aroma of the product and that some of these bodies are sufficiently different from vanillin and coumarin in their behavior to be unextractable by ether.

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## BOOK REVIEWS

**Statistical Methods Applied to Experiments in Agriculture and Biology.** By GEORGE W. SNEDECOR. The Collegiate Press, Inc., Ames, Iowa. Third Edition (1940). XIII + 422 pp. Price \$3.75.

Although a review of the Second Edition of this book was given in *This Journal*, 22, 196 (1939), it is worth mentioning again, especially in view of the fact that new material has been added.

This book is one of the very good texts on statistics for the beginner. It deals primarily with methods applied to experiments in agriculture and biology. It is very readable, though sometimes the explanations seem lengthy, and detailed discussions of statistical procedures are perhaps unnecessarily given. The author emphasizes the use of common sense in statistics and the fact that hypotheses should be formed upon consultation with the biologist, or agricultural experimenter, and not because the numbers fall in a certain pattern in that particular sample. One interesting section heading is "Planned Experiments and Those That 'Jes' Grewed'." Very little theoretical statistics is given, and the publication is essentially a beginner's text rather than a reference book. In fact, many more classic references could profitably be included with less emphasis on publications by Iowa State College.

Analyses of variance and covariance are covered quite thoroughly, and much emphasis is placed on the fiducial limits and tests of significance, especially on the chi-square test. Linear regression, correlation, and multiple and curvilinear regression are also covered rather thoroughly. One chapter is devoted to the binomial and Poisson distributions.

Tables and examples are numbered according to the chapter in which they appear, for example, Table 10.2 would be the second table in Chapter 10.

In this edition new developments in methodology have been included, such as: a test for homogeneity of variance in groups that have unequal numbers; analysis of variance in regression; and three types of transformations of data (square root, logarithmic, and arc sine  $\sqrt{\text{percentage}}$ , including a table for the latter). There is also a new chapter on sampling that gives three objectives of sampling, how the size of a sample should be theoretically determined, an example of analysis of data from two or more populations, and a section on the structure of sampling investigations. This chapter is a valuable addition to the book.

As stated previously, this text is excellent for the novice and should help build a strong foundation for the intelligent use of statistics.—LILA F. KNUDSEN.

**The Scientific Principles of Plant Protection.** With Special Reference to Chemical Control. Third edition. By HUBERT MARTIN. Longmans, Green & Co., New York, and Edward Arnold and Company, London, 1940. Price \$7.00.

This edition follows very closely the pattern of the previous edition, which was reviewed in *This Journal*, 20, 332 (1937). It is a study of the factors that affect plant protection, including natural controls, plant resistance, the effects of soil, climate and cultural methods, biological control of insects and plant diseases, insecticides and fungicides with their adjuncts, and weed killers.

Some new material necessitated by research, published since the last edition, has been included, particularly in the sections dealing with insecticides and fungicides. Additional information on copper fungicides and aliphatic thiocyanates has been incorporated and a new section on dinitrophenols added. However, most of the material is similar to that in the previous edition.—E. L. GRIFFIN.



**The Chemical Constitution of Natural Fats.** By T. P. HILDITCH. John Wiley and Sons, Inc., New York. 1940. XI+438 pp., 113 tables, 11 figures. Price \$6.50.

Our knowledge of the constitution of natural triglycerides has greatly increased since 1927, largely due to the work of Professor Hilditch and his collaborators at the University of Liverpool. In this book, the newer concepts resulting from these fundamental investigations, together with a large amount of recent data, are presented adequately in English for the first time. The volume accomplishes the objective of the author, which is "to present as complete a statement as possible of the existing knowledge of the chemical constitution of natural fatty compounds, especially the glycerides."

To the chemist familiar with the well-known monographs on fats, this book offers several unusual features. The distribution and composition of the natural triglycerides and their component fatty acids are discussed almost exclusively. The usual "constants" and methods employed in the analysis of fatty materials are mentioned only incidentally. In place of the familiar arbitrary groupings of the fats, such as those depending upon iodine absorption, there is a more logical classification based on the modern data that discloses a close relationship between the component acids and the biological origin of the fat.

The rather extended introduction (Chapter I) summarizes the author's motives for adopting this biological classification and presents a preliminary survey of the material considered in this book. The acids found in the fats of aquatic and land animals and in vegetables are discussed in great detail in the next three chapters, illustrated by numerous tables giving the results of modern investigations. After a short chapter reviewing the scanty information on natural glycerides acquired prior to 1927 (Chapter V), the component glycerides of vegetable and animal fats are described in Chapters VI and VII. Chapter VIII indicates briefly the application of the newer knowledge of the chemical constitution of natural fats to various biochemical problems. After two comprehensive chapters on the constitution and properties of fatty acids, alcohols, and synthetic glycerides, the book concludes with a useful chapter on the modern methods used in the quantitative study of the constitution of fats. This last chapter includes examples of the calculations used in the interpretation of the experimental data. The value of this book is greatly enhanced by the numerous references at the end of each chapter. Adequate indexes of general subjects, individual fats, fatty acids, and glycerides are provided.

The book is attractively printed, strikingly free from typographical errors, and conveniently outlined by abundant section headings. The presentation is clear and well written, although there is some tendency towards repetition. This work will be particularly valuable to the research specialist in the field of fats and it undoubtedly will "stimulate research, draw the attention of investigators to what has already been done and to the lacunae which still exist." Those who are not primarily concerned with research in this field will find this book useful for its data and references.—J. FITELSON.

**Review of Chemical Species.** By JEAN TIMMERMANS. Translated from the revised French Manuscript of *La Notion d'Espece en Chimie*, published at Paris in 1928, by RALPH E. OESPER. VIII+177 pp. Chemical Publishing Co., Inc., New York, N. Y., 1940. Price \$4.00.

This book, by the Director of the International Bureau of Physical Chemical Sciences at Brussels since its creation in 1921, was originally published in Paris in 1928. Some additions and alterations have been made in the revised issue in English, but citations to the literature subsequent to 1925 are infrequent. References are generally to European publications.

The stated scope of this little volume of 177 pages comprises "an examination of the most favorable conditions for the determination of physical chemical constants." The treatment is admittedly "neither complete nor exhaustive." The book is divided into four main parts. Part I (48 pages) deals with the criteria that may be applied to define precisely a physico-chemical system. That is the problem of chemical species. Part II (49 pages) deals with some methods of preparation of pure materials and some criteria of purity. A material is defined as pure "if it is perfectly homogeneous after it has been subjected to successive modes of fractionation that are as different as possible." This definition is elaborated, and it is recognized that the purity of a material is a relative concept, differing according to the purpose in view. Part III (39 pages) deals with methods of determining physical constants. It includes a consideration of units, standards, the nature of experimental errors, and a brief chapter dealing with the theory of least squares. Part IV (29 pages) is entitled "The way to find in the literature the best method of purification and the most probable value of the constants of pure materials. Examples and applications." The discussion indicates some of the specialized laboratories in which exact determinations of physical constants are made, and where collections of physical constants are published. Criteria for critical appraisal and choice of published data are also given.

The book is very uneven in scope and adequacy of treatment. Thus, reference is made to chromatographic adsorption as a method of purification, with literature citations to 1939, but although two chapters are devoted to fractional distillation ("the most effective method of fractionation"), they contain only two references to data published after 1924, both to the same author. In neither chapter is there reference to any American literature on the subject despite the tremendous advances in the development of this tool in recent years, especially in the United States. The concept of theoretical plates is not even referred to.

The best feature of the book is the large number of apt examples illustrating typical difficulties, traps for the unwary, and solutions to problems that frequently confront every chemist. Consideration of these classic examples and due regard for the principles enunciated in this little volume would do much to relieve the literature of descriptions of numerous compounds that never existed and the superabundance of physical constants on materials of questionable purity.

The print is good and apparently free from typographical errors, but the figures are frequently poor and the legends sometimes nearly illegible. There is no index.—L. A. GOLDBLATT.

**Qualitative Analysis and Chemical Equilibrium.** By T. R. HOGNESS and WARREN C. JOHNSON. Revised. 538 pp. Henry Holt and Co., New York, N. Y. 1940.

The book is divided into two parts, the first part containing nine chapters and the second ten chapters. There is also an appendix of 65 pages.

Part I deals with the theoretical and physico-chemical explanations of the phenomena encountered in qualitative analysis, and includes chapters on reaction velocities, equilibria, ionization, hydrolysis, oxidation and reduction, solubility product, and amphoteric substances. Each chapter includes a list of questions and problems relating to the principles explained in the text.

Part II gives a description of the common laboratory operations and general instructions for qualitative analysis. Detailed instructions are given for the separation of each of the groups and for the identification of each of the elements contained in the group. Certain changes have been made in the revised edition and their advantages are explained. Characteristic reactions and properties of each of the metallic ions are also explained.

The authors state that, because of the recent appearance of satisfactory ap-

paratus for centrifugation processes, they have in this edition designed the laboratory procedures for both centrifugation and filtration processes. "Wherever necessary we have split the page into two vertically divided parts; the left part referring to centrifugation, the right to filtration."

Emphasis is placed on the fact that smaller amounts of solution and more dilute concentrations are advisable from the standpoints of economy of time and reliability of results.

The methods used in the preparation of samples are outlined.

There are two chapters outlining the method for the identification of the negative ions and giving a description of their properties. A series of elimination tests is also given for the detection of anions.

The appendix gives a list of apparatus and reagents, mathematical operations, ionization constants, answers to problems given in the text, and a table of the properties of compounds. This information regarding the properties of the compounds would perhaps be more readily available if it were arranged alphabetically rather than in the order of the appearance of the elements in the qualitative scheme.—  
ROSCOE H. CARTER.

**Calcium Superphosphate and Compound Fertilizers. Their Chemistry and Manufacture.** By P. PARISH and A. OGILVIE, with forewords by Dr. H. C. BROWN and WALTER G. T. PACKARD. Distributor, Chemical Publishing Co. Inc. New York. Pages xiii+322. 152 figures. Price \$14.00.

The book represents an extensive revision of *Artificial Fertilizers: Their Chemistry, Manufacture and Application* published more than twelve years ago. Six new chapters have been introduced.

The subjects discussed are (1) General survey of the fertilizer position, particularly in regard to superphosphate, (2) World's phosphate supply, (3) History and chemistry of the manufacture of soluble phosphates, (4) Crushing and grinding plant, (5) Manufacture of calcium superphosphate-mixing plant, (6) Mechanical dens and excavators, Batch type and continuous type, (7) The Oberphos process, (8) Drying of superphosphate, (9) Methods of handling toxic gases, (10) Treatment and storage of superphosphate, (11) Compound manures and mixing systems, (12) Manufacture of phosphoric acid and superphosphate, (13) Manufacture of bone superphosphate, (14) Manufacture of basic slag, (15) Mechanical handling of raw materials, (16) Crystallization and granulation of fertilizer products, (17) The British superphosphate industry and the design and management of a superphosphate works.

The book is well written and contains many excellent illustrations.—H. R. KRAYBILL.

## TUESDAY—MORNING AND AFTERNOON SESSIONS

### REPORT ON PLANTS

By E. J. MILLER (Agricultural Experiment Station,  
East Lansing, Mich.), *Referee*

In connection with the apparent inconsistency in the program on plants, in that the Referee on Boron has no report, while the Referee on Iodine is presenting a report on boron, it may be stated that at the time of the meeting last year the Referee had no knowledge that Dr. McHargue was still active in work on boron, but did know that Dr. Cook was actively engaged in work on methods for boron and therefore recommended his appointment as Associate Referee on Boron. On receipt of a copy of Dr. McHargue's report on boron, the Referee discussed the matter with Dr. Cook, and he stated that perhaps it would better serve the interest of the work to have Dr. McHargue submit the method for collaborative study and he (Dr. Cook) would be glad to collaborate.

Work on methods for copper and cobalt has progressed, and it is hoped that substantial contributions can be made soon.

In connection with the work on chlorophyl and carotene, the keen interest in the methods studied and reported in *This Journal*, 23, 709-16 (1940) makes further study urgent. The Referee hopes that official methods may result therefrom.

The Associate Referee on Zinc in Plants has had considerable correspondence with the Associate Referee on Copper and Zinc in Soils and Fertilizers, W. Y. Gary, and as a result of their collaboration on the method for zinc in plants the possibility of adopting it for fertilizers is being considered.

The Associate Referee on Zinc in Plants has also worked out an excellent method for iron in plants, which it is hoped can be tested in collaborative work in the coming year, and it is therefore recommended that he be appointed Associate Referee on Iron also.

The excellent accomplishments of the several associate referees on plants and their collaborators in the past year are gratifying and greatly appreciated.

### RECOMMENDATIONS\*

It is recommended—

(1) That studies on iodine be continued; that the associate referee, J. S. McHargue, be appointed Referee on Boron also; and that collaborative studies on the boron method be initiated.

(2) That work on carbohydrates be continued.

(3) That the recommendations of the Associate Referee on Zinc be adopted.

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\* For report on Subcommittee A and action by the Association, see *This Journal*, 24, 48 (1941).

(4) That the Associate Referee on Zinc be authorized to continue a study of a method for iron, and if circumstances warrant initiate collaborative work.

(5) That studies on copper and cobalt be continued.

(6) That the present Associate Referee on Boron be relieved of his duties as indicated previously.

(7) That the recommendations of the Associate Referee on Chlorophyll and Carotene be adopted.

(8) That studies on hydrocyanic acid be continued.

## REPORT ON IODINE AND BORON\*

By J. S. MCHARGUE, *Associate Referee*, and W. S. HODGKISS  
(Department of Chemistry, Agricultural Experiment  
Station, Lexington, Ky.)

During the past year attention was given to methods for the determination of boron in soils, plants, and animal tissue, but no study of iodine was made. This report is primarily concerned with the determination of boron in various kinds of plant tissue. Several methods were tried, but the quinalizarin procedure described by Berger and Truog,<sup>1</sup> with some slight modifications,<sup>2</sup> was finally used. The procedure carried out by Hodgkiss is described, and tables containing the data obtained are presented.

### BORON IN PLANT MATERIAL

#### REAGENTS

(1) *Sulfuric acid*.—0.36 *N* (approximate). 10 ml. of 96%  $\text{H}_2\text{SO}_4$  per liter.

(2) *Quinalizarin—sulfuric acid indicator*.—98.5% by weight of  $\text{H}_2\text{SO}_4$  containing 0.008 gram of quinalizarin per liter.

(3) *Standard boron solution*.—1.1432 grams of  $\text{H}_3\text{BO}_3$  per liter of 0.18 *N*  $\text{H}_2\text{SO}_4$ . Dilute so that a series of standards contains from 0 to 0.005 mg. of boron per ml. in 0.18 *N*  $\text{H}_2\text{SO}_4$ .

#### PROCEDURE

Ignite a 0.25–1.00 gram sample of the plant material to a white or gray ash at 450° C. in either porcelain or platinum vessels. After cooling dissolve the ash in 5 ml. of the  $\text{H}_2\text{SO}_4$  and mix thoroughly. Allow residue to settle or centrifuge to clarify; take a 1 ml. aliquot of the clear supernatant liquid and transfer to a comparison tube. Add 9 ml. of the acid-indicator solution and mix thoroughly. Stopper, and allow to stand for 30 minutes before making a comparison with standards either visually or by the use of a photoelectric colorimeter.

### OBSERVATIONS ON THE PROCEDURE

The sensitivity of the method is dependent upon the final acid and indicator concentration of the comparison solution. Since a maximum sensi-

\* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 540 (1939)

<sup>2</sup> Hodgkiss, W. S., McHargue, J. S., and Offutt, E. B. Presented at the meeting of the American Chemical Society, April, 1940.

tivity appears at approximately 92 per cent (1) of sulfuric acid, an adjustment of acid volume added to the aliquot of the unknown will bring the sensitivity to the desired level. An increased concentration of quinalizarin will increase the usable spread of the standard curve but will somewhat reduce the sensitivity.

Owing to the absorption of moisture by the mixed acid-indicator solution, the standard curve will change slightly with time although it will remain parallel to the previous one.

### EXPERIMENTAL

The use of a base to prevent loss of boron during ashing at the temperature stated previously was not found to be necessary. Table 1 shows the variations found on ashing several samples containing a wide range of boron concentration.

TABLE 1.—*Boron in plant material ashed with and without  $MgO_2$  and  $K_2CO_3$*

SAMPLE	NO ADDITION	$MgCO_3$	$K_2CO_3$
Tobacco seed	6.1	5.9	6.0
Hickory stem	9.3	9.0	9.4
Hickory petiole	15.1	14.6	15.4
Sumac leaves	19.3	19.3	19.3
Hickory leaves	25.7	23.8	26.0
Alfalfa	33.5	33.5	33.8
Hickory leaves	40.9	39.2	41.0

A 2 to 1 proportion of magnesium peroxide and plant material was used in one case, while a solution equivalent to 0.4 gram of potassium carbonate was used in the other. The variations in the separate analyses come well within the experimental error of the colorimetric procedure.

Table 2 shows the reproducibility of the procedure in the analysis of plant material. Duplicate aliquots taken from the same solution of the ash were compared to duplicates from a second ashing of another portion of the same sample. The table also shows the variations found on duplicate ashings of a series of samples that differed considerably in their boron content.

In general there is good agreement between the duplicates from the same solution of ash, but a somewhat greater variation is shown between determinations made on duplicate ashings. The greater variation in the latter case is probably due to the lack of homogeneity of the original sample, especially when a sample as small as 0.25 gram was taken for analysis.

The boron determinations made on various plant materials show the ranges given in Table 3. Preliminary spectrographic analyses for boron on these same plant materials showed good correlation between that procedure and the quinalizarin-colorimetric method.

TABLE 2.—*Reproducibility of results from boron determinations on plant material (p.p.m. boron of moisture-free material)*

SAMPLE NO.	1ST ASHING			2ND ASHING			MEAN OF DUPLICATED ASHINGS
	A	B	MEAN	A	B	MEAN	
507	0.39	0.43	0.41	0.45	0.43	0.44	0.43
508	0.27	0.27	0.27	0.32	0.34	0.33	0.30
509	2.55	2.37	2.46	2.72	2.58	2.65	2.56
510	0.64	0.64	0.64	0.74	0.74	0.74	0.69
511	0.78	0.74	0.76	0.64	0.62	0.63	0.70
512	0.82	0.86	0.84	0.93	0.95	0.94	0.89
785	2.95	2.90	2.93	2.89	2.87	2.88	2.90
786	3.00	3.00	3.00	2.98	3.03	3.00	3.00
788	52.5	51.5	52.0	52.5	53.5	53.0	52.5
789	67.0	67.0	67.0	68.5	68.0	68.3	67.6
790	21.5	21.3	21.4	23.6	23.8	23.7	22.4

TABLE 3.—*Boron content of various plant materials (p.p.m. of moisture-free material)*

MATERIAL	NUMBER SAMPLES	RANGE	AVERAGE
Cereals	10	0.6 – 2.3	1.2
Pasture and hay crops	8	3.30– 11.8	6.6
Leguminous hays	5	18 – 47	30
Vegetables	4	4.4 – 41	22
Fruits	10	8 – 38	20
Nuts	5	2 – 24	12
Leaves	20	19 –160	60
Petioles	7	11 – 19	16
Stems	5	9 – 16	12

The results show that the quinalizarin method for the determination of boron in plant materials is quite satisfactory. Some objections may be made because of the very strong sulfuric acid required, but the speed and simplicity of the procedure should offset such objections.

No report on carbohydrates was given by the associate referee.

### REPORT ON ZINC IN PLANTS\*

By HALE COWLING (Chemical Section, Agricultural Experiment Station, East Lansing, Mich.†), *Associate Referee*

Before the appointment of the present Associate Referee on Zinc in Plants he already had an investigation well underway for the purpose of

\* Published with the permission of the Director of the Experiment Station as Journal Article No. 48. (n. s.).

† Present address: American Viscose Corp., Marcus Hook, Pa.

developing a more sensitive and accurate method for the determination of zinc in plants than is possible by the present A.O.A.C. method, *Methods of Analysis, A.O.A.C.*, 1940, 131. This work resulted in the development of a photometric dithizone method that proved to be accurate and remarkably free of interferences by other metals that form colored complexes with dithizone. The details of the research leading to the development of the method and the experiments that were carried out to prove the method to be reliable have been published,<sup>1</sup> and for this reason will not be included here.

After the method had been thoroughly tested, it was submitted with the results obtained to the Referee on Plants, who advised that the method be studied collaboratively. About 20 persons were contacted, but only 7 consented to participate in this collaborative study; later 2 of these requested that they be relieved of the work, and a third was not able to complete the work in time for inclusion in this report. In Table 1 are given

TABLE 1.—*Collaborators*

COLLABORATOR	ANALYSES MADE BY:	LOCATION
1. H. R. Kraybill	Harold Nash D. M. Doty	Purdue University Agricultural Experiment Station
2. J. Davidson	Ben L. Kaspin	U. S. Department of Agriculture Bureau of Agricultural Chemistry and Engineering
3. Horace G. Byers	Kenneth C. Beeson	U. S. Department of Agriculture Bureau of Plant Industry
4. W. Y. Gary	W. Y. Gary	State of Florida Agricultural Department Chemical Division

the names of the collaborators and also of those that actually carried out the analyses. To these persons is extended an expression of sincere appreciation for their efforts, which have made this report possible.

Three samples of plant materials were submitted to each collaborator. Sample 1 was alfalfa leaf meal, Sample 2 was dried parsnip roots, and Sample 3 was dried spinach. All samples were finely ground to insure uniformity. Each collaborator was furnished a description of the method to be used, the actual procedures to be followed, and a brief statement regarding the principles of the method and the results the Associate Referee had obtained.

#### COLLABORATIVE RESULTS

Each collaborator was asked to apply the ashing procedure to two samples of each plant material, and to make duplicate determinations on

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 13, 145 (1941).



each of the ash solutions obtained. The results obtained by the collaborators are given in Table 2.

TABLE 2.—*Collaborative results on zinc in plant materials*

COLLABORATOR	ASH SOLUTION	ZINC FOUND (P.P.M.)		
		SAMPLE 1	SAMPLE 2	SAMPLE 3
1	A	26.4-26.6	22.4-22.4	48.8-49.4
	B	26.4-26.2	22.4-22.0	49.2-49.6
2	A	24.4-24.4	18.6-19.4	49.4-50.4
	B	24.4-25.0	20.0-20.6	49.2-49.0
3	A	21-22	18-17	47-48
	B	20-21	18-18	43-45
4	A	30-26	26-24	42-42
	B	24-26	22-22	42-44

In order to obtain values for comparison with those obtained by the collaborators, the Associate Referee analyzed three samples twelve times. Three ashings of each sample were carried out simultaneously, and the determination was made on one aliquot from each ash solution obtained. When the value obtained by analysis of the first aliquot did not agree well with previously obtained results (Ash Solutions 7, 8, and 9 for Samples 1 and 3) analyses were repeated on a second aliquot of the ash solution. The results obtained by the Associate Referee on the referee samples are given in Table 3.

TABLE 3.—*Results of analyses of referee samples by associate referee*

ASH SOLUTION	ZINC FOUND (P.P.M.)		
	SAMPLE 1	SAMPLE 2	SAMPLE 3
1	25.6	22.8	47.6
2	26.0	22.4	46.0
3	26.0	22.6	47.8
4	25.4	22.2	46.4
5	25.0	21.8	47.6
6	25.4	21.7	47.4
7	24.6-24.5	22.4	49.6-49.6
8	27.6-27.6	22.0	44.0-44.2
9	23.9-24.4	22.3	47.4-47.8
10	23.7	22.8	47.6
11	24.9	22.2	45.8
12	24.7	22.2	45.0
Average	25.3	22.3	46.9

For purposes of comparison the average quantities of zinc found by the collaborators and by the Associate Referee for each of the samples are compiled in Table 4.

TABLE 4.—*Comparison of averages*

COLLABORATOR	SAMPLE 1	SAMPLE 2	SAMPLE 3
1	26.4	22.3	49.3
2	24.6	19.7	49.5
3	21.0	17.8	45.8
4	26.5	23.5	42.5
(Associate Referee)	25.3	22.3	46.9

It was requested that each collaborator furnish certain pertinent data on the instruments used in this study. This information is given in Table 5.

TABLE 5.—*Photoelectric colorimeters used*

COLLABORATOR	INSTRUMENT	CELL THICKNESS	FILTER USED	DILUTION OF FINAL EXTRACT
1	KWSZ	1.0 cm.	Corning No. 401 (3.73 mm.)	5-25 ml.
2	Evelyn	0.75 in. (circular)	Evelyn 540	5-50 ml.
3	Evelyn	2.2 cm.	Evelyn 520	5-50 ml.
4	Fisher	1.0 cm	Green Wratten	5-25 ml
No. 7-089				
Associate Referee	Cenco-Sheard-Sanford	1.0 cm.	Corning No. 401 (6.0 mm.)	5-25 ml.

Collaborators were encouraged to comment on the method and their experiences with it. The following comments were received.

## COMMENTS OF COLLABORATORS

*H. R. Kraybill.*—Some difficulty was experienced with fading in ordinary diffused sunlight. Sufficient fading took place between the development of color in the final extraction and the last reading on the photometer to introduce errors of as much as 6%. When the photometer was moved to the dark room, where electric light supplied the only illumination, fading was overcome. In fact, a degree of intensification of color was evidenced during at least the first 2 hours under these conditions. No change in color intensity was encountered after 48 hours in absolute darkness.

Sample 3 seemed difficult to ash, and the ashing solution obtained filtered very slowly. Some suspended material was evident in the ashing solution after it had been made to volume and acidified. The KWSZ photometer used was equipped with gas-filled photocells and 45 volts was used on the galvanometer. The standard curve prepared has zinc concentration plotted against the log values of the absorption. We found this way better than to plot concentration directly against transmission as suggested in your directions. The color developed does not follow Beer's law as shown by the fact that the standard curve is not a straight line. The method as ap-

plied here has an accuracy of  $\pm 1\%$ , which is very good for this type of determination.

*J. Davidson.*—Readings were obtained on the Evelyn, also on a Brice photoelectric colorimeter. The Brice readings agreed quite well with those obtained on the Evelyn but were somewhat less consistent. Only the results obtained with the Evelyn are recorded on the accompanying form.

The instructions were followed very closely. They were found to be comprehensive and clear. For the extraction of the ash of material No. 3, 10 ml. of normal HCl proved to be insufficient, and 25 ml. was used.

*H. G. Byers.*—The reagent blank treated in exactly the same way as the unknown samples gave the same readings as the standard sample that contained no zinc and which was used to obtain the point of zero absorption on the standard curve. No "blank correction" was therefore made. Sample 3 seemed to be difficult to ash properly. No other difficulties were encountered, and the results seemed to be easily reproducible.

*W. Y. Gary.*—I think this is a very fine method. It also works well for zinc in fertilizers up to 0.50%. I ashed 1 gram of the fertilizer and made up the solution so that a 10 ml. aliquot came within the range of the method. Some of the precautions may not be necessary; e.g., 9.6 ml. of our regular distilled water produced the same blank as that obtained for the zero point in the standards. From the quantities of HCl and  $\text{NH}_4\text{OH}$  required, the guaranteed analyses on our C. P. reagents indicate that they do not need redistillation.

#### DISCUSSION OF RESULTS

The results obtained by the collaborators and by the Associate Referee (Tables 2 and 3) agree well for duplicate determinations on the same ash solution. However Table 4 shows that although Collaborator 1 and the Associate Referee obtained values that agree closely for all three samples, on the whole the variations among all collaborators are quite appreciable. It should be borne in mind that dithizone extractions involve technics unfamiliar to most analysts, and in some instances this was probably the case in this study.

Some of the zinc in the plant ash may be rather difficult to extract with hydrochloric acid when the amount of material insoluble in hydrochloric acid in the plant ash is high. This might be the case for Sample 3, the ash from which contained a large quantity of silica, and least in the case of Sample 2, the ash of which was practically all soluble in hydrochloric acid. Variations in the time of heating after the addition of hydrochloric acid might result in more complete extraction of the zinc in some cases than in others, and cause variations in results. It is believed that this problem of the extraction of the zinc from the plant ash is of sufficient importance to warrant future study.

The observation mentioned in the comments of Kraybill concerning the fading of the color of the final extract in diffused sunlight and the absence of fading in artificial light is important. It is well known that dithizone complexes in general are subject to fading in sunlight, and the description of the method that was sent to the collaborators should have contained a precaution to this effect, but this was overlooked. Perhaps working in a

room illuminated solely by electric lights, as suggested by Kraybill, is as convenient a way as any for avoiding errors due to fading.

After the collaborative study was well under way it was found that the lot of dithizone that had been used in the development of this method was much less pure than a second lot, which had just been obtained, although both lots were purchased after the manufacturer had announced an improvement in the purity of the product. The result was that the preparation of the dithizone according to the directions given resulted in a reagent about twice as concentrated as was the case when the first lot was used. It was evident that all collaborators used very pure products in their work, and as a result their dithizone reagents were more concentrated than was intended. This should not have affected their results to any appreciable extent, but it would tend to reduce the sensitivity of the photoelectric colorimeter to changes in zinc concentration.

The Associate Referee considers that this new method for the determination of zinc in plants has several distinct advantages over the present A.O.A.C. method. It requires an aliquot of plant ash solution equivalent to 0.5 gram instead of 25 grams, and it is more rapid if a large number of samples is to be analyzed. Experience has shown that six samples may be taken from the point of ash solution to the final result in two and a half hours, which is equivalent to less than 30 minutes for each determination. It is believed that the method will give results of greater accuracy than does the present method after the analyst has mastered the technic. The method, however, should be tested further before it is recommended as an official A.O.A.C. method.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method suggested be adopted as tentative pending further investigation. The method presented to the collaborators differed from the text published in *This Journal*, 24, 71 (1941), in the two following particulars: 0.30 gram of the dithizone reagent was dissolved instead of 0.20 gram, and after the first paragraph of the description of the procedure for the final extraction, the following caution was inserted: "After the final extract is obtained it should be exposed only to electric light until after photoelectric colorimeter readings have been taken."

(2) That the method be further studied and particular attention be given to the ashing of the sample and extraction of the zinc from the plant ash.

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No report on copper and cobalt was given by the associate referee.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 59 (1941).

No report on boron was given by the associate referee.

## REPORT ON CHLOROPHYL AND CAROTENE IN PLANT TISSUE\*

By ERWIN J. BENNE (Agricultural Experiment Station,  
East Lansing, Mich.), *Associate Referee*

The interest of increasing numbers of investigators in carotene and chlorophyll urges the eventual inclusion of practicable methods for their evaluation in plant tissues in *Methods of Analysis*, A.O.A.C.

Although the Associate Referee is unaware of any collaborative work having been undertaken by the Association on methods for determining chlorophyll, an important step in this direction so far as carotene is concerned was taken by Munsey and his collaborators,<sup>1</sup> who compared the Peterson-Hughes-Freeman modification of the Guilbert method,<sup>2</sup> with a number of procedures in current use, and as a result of their study recommended it with some modifications as a tentative method for evaluating carotene in feeding stuffs, many of which obviously consist of plant materials.

A brief résumé of a few recent articles, however, will show that there have been proposed for the evaluation of carotene in plant materials, numerous new or modified procedures that appear of sufficient value to warrant further collaborative study, since they claim to effect more thorough separation of other pigment impurities, to lessen manipulative details, or to overcome difficulties presented by individual plant tissues. In general, the same is true of chlorophyll, although less work has been done on methods for evaluating this pigment.

Buxton and Dumbrow<sup>3</sup> replace petroleum benzin or Skellysolve as used in the Peterson-Hughes-Freeman procedure with technical heptane, and give an extinction coefficient for beta carotene in this solvent to be used with the spectrophotometer at 4500Å. Shrewsbury, Kraybill, and Withrow<sup>4</sup> report values of specific absorption coefficients at different wave lengths for use with the photoelectric photometer and spectrophotometer in evaluating concentrations of alpha and beta carotene in the same solvent. Wiseman, Kane, Shinn, and Cary,<sup>5</sup> reporting on the carotene content of market hays and corn silage, describe methods that they consider particularly suited for preparing and analyzing samples of such materials.

Sherman and Salmon<sup>6</sup> report using successfully a method somewhat similar to the Peterson-Hughes-Freeman technic, but in which a solution

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<sup>1</sup> *This Journal*, 20, 459 (1937); 21, 626 (1938); 22, 664 (1939).

<sup>2</sup> *Ind. Eng. Chem., Anal. Ed.*, 9, 71 (1937).

<sup>3</sup> *Ibid.*, 10, 262 (1938).

<sup>4</sup> *Ibid.*, 253.

<sup>5</sup> *J. Agr. Research*, 57, 635 (1938).

<sup>6</sup> *Food Research*, 4, 371 (1939).

of potassium hydroxide in methanol is substituted for potassium hydroxide in ethanol, for determining the carotene content of soybeans and cowpeas. Sherman<sup>7</sup> later made chromatographic analyses and bioassays of the biologically active carotenoid pigments extracted from mature soybean seeds, and concluded that the pigment in the purified extracts consisted of 80–88.8 per cent beta carotene and 2.5–11 per cent alpha carotene, with no cryptoxanthin present. Koehn and Sherman<sup>8</sup> and Moore<sup>9</sup> are among those to report recently the successful use of photoelectric colorimeters for the routine evaluation of carotene in hydrocarbon solvents.

Hegsted, Porter and Peterson<sup>10</sup> report that the use of diacetone in place of the usual 90 per cent methanol or 85 per cent ethanol for removal of pigments other than carotene from Skellysolve or petroleum benzin results in an improved method for determining the carotene content of silages, as shown by comparison with carotene contents determined by chromatographic technics. Zimmerman, Tressler and Maynard<sup>11</sup> report the use of a modified form of the above method for determining carotene, in which they use hot diacetone to extract total pigments from fresh and frozen vegetables. Subsequent dilution with water causes the retention of most of the non-carotene pigments when carotene is extracted with petroleum benzin, from which the small amounts of dissolved chlorophyll and xanthophyll are removed with properly diluted diacetone and water after saponification of the former with methanolic potassium hydroxide.

Fraps and Kemmerer<sup>12</sup> and Fraps, Kemmerer, and Greenberg<sup>13</sup> are among those that contend that non-carotene pigments can best be separated from carotene dissolved in petroleum hydrocarbons by use of some type of adsorption technic, and report upon the use of magnesium oxide and magnesium carbonate for this purpose, although preparation of successive lots of these materials that do not also absorb some carotene has been admittedly difficult. Moore<sup>14</sup> reports the successful use of dicalcium phosphate as a chromatographic adsorbent for separating non-carotene pigment impurities from petroleum hydrocarbon solutions of carotene.

Lease and Mitchell<sup>15</sup> state that methods for determining carotene involving the use of alcoholic potassium hydroxide were found inapplicable to cooked sweet potatoes, stored raw sweet potatoes, and certain other cooked vegetables, apparently because polymerization of carbohydrates by the alkali formed a resinous film, rendering the carotene unextractable by cold or boiling 95 per cent ethanol, ether, acetone, or petroleum benzin. Carotene in such materials can be determined by extraction with ethanol,

<sup>7</sup> *Food Research*, 5, 13 (1940).

<sup>8</sup> *J. Biol. Chem.*, 132, 527 (1940).

<sup>9</sup> *J. Dairy Sci.*, 22, 501 (1939).

<sup>10</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 256 (1939).

<sup>11</sup> *Food Research*, 6, 57 (1941).

<sup>12</sup> *This Journal*, 22, 190 (1939).

<sup>13</sup> *Ibid.*, 23, 422, 659, (1940); *Ind. Eng. Chem., Anal. Ed.*, 12, 16 (1940).

<sup>14</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 726, 1940.

<sup>15</sup> *Ibid.*, 337.

but if necessary to use alcoholic potassium hydroxide, they should be subsequently boiled with water to remove the resins before extraction of carotene by petroleum hydrocarbon fat solvents. In this connection Moon<sup>16</sup> asserts that alkaline extracts of grass contain resinous substances which, in the presence of alcohol are precipitated in close contact with the substance being analyzed, thus making subsequent extraction of carotene difficult. In order to overcome this he suggests two alternatives: (1) Extraction of the grass with hot alcohol, followed by treatment of the extract with potash; or (2) saponification with hot aqueous potash, followed by filtration and extraction of the grass residue with alcohol.

Johnston and Weintraub<sup>17</sup> present a method for determining chlorophyll in acetone extracts of plant tissue without removal of other pigments; however, the special apparatus involved makes the procedure less applicable for general use than one utilizing an ordinary photoelectric colorimeter.

Mackinney<sup>18</sup> reports on methods for extracting chlorophyll from plant tissue and for separating and purifying a and b chlorophyll, and gives criteria for judging the purity of such preparations.

Petering, Wolman and Hibbard<sup>19</sup> describe a method of more than usual interest to investigators concerned with determining both carotene and chlorophyll. A photoelectric colorimeter equipped with suitable light filters is used to evaluate chlorophyll in acetone extracts of plant tissue without removal of other pigments, and later to evaluate carotene in a petroleum benzin extract of an aliquot of the same solution after chlorophyll is removed by the apparently specific action of barium hydroxide toward this pigment. Xanthophyll and flavones are separated from the petroleum benzin solution of carotene by the usual procedures, and a means of correcting for light absorption by traces of chlorophyll, if retained in this solution, is presented. Benne, Wolman, Hibbard, and Miller<sup>20</sup> compared carotene values obtained by this method and by two modifications thereof for determining carotene only, with those obtained by the Peterson-Hughes-Freeman technic on a variety of plant tissues, and found satisfactory agreement in nearly all cases.

Thus there is ample recent material upon which to draw for a collaborative study of methods for determining these constituents of plant tissue. Indeed it appeared impossible to include all the available procedures without making the task prohibitively burdensome for individual collaborators. In an effort to avoid this and still include as many worthwhile suggestions as possible, letters were sent to many that had recently suggested new or modified methods, as well as to some others, asking if they would care to participate in such a study, in the hope that each would

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<sup>16</sup> *J. Agr. Science*, 29, 295 (1939).

<sup>17</sup> *Smithsonian Misc. Col.* 98, No. 19 (1939).

<sup>18</sup> *J. Biol. Chem.*, 132, 91 (1940).

<sup>19</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 148 (1940).

<sup>20</sup> *This Journal*, 23, 709 (1940).

consent to use his special technic on collaborative samples, and in this way make available to those interested a comparison of results in the published report. A number sufficient to make collaborative work profitable agreed to take part, and the study was undertaken with the approval of the Referee on Plants.

#### DIRECTIONS TO COLLABORATORS

In initiating this study two samples of finely ground plant tissue, alfalfa meal and spinach leaves, were furnished each collaborator (6-27-40), together with reprints, mimeographed material, or references to aid them in following the instructions given. For carotene it seemed advisable to use the Peterson-Hughes-Freeman technic,<sup>2</sup> which as mentioned previously has been recommended in its essential features as a tentative A.O.A.C. method, as a standard, and to compare the other procedures with it. Since the Petering-Wolman-Hibbard<sup>19</sup> method includes means of evaluating both pigments and separates chlorophyll without the conventional use of a soluble alkali, and since one of its modifications for determining carotene only<sup>20</sup> appeared to make possible a worthwhile saving of time, it was decided to ask the collaborators to use these technics, and any others that they cared to, upon each of the collaborative samples.

The collaborators were given their choice of working with either or both of the pigments. Those who were to work on chlorophyll were asked to use the Petering-Wolman-Hibbard method, and any other procedures they cared to, on the collaborative samples. Each was furnished a sample of 5X chlorophyll, purchased from American Chlorophyll, Inc., in order that a common primary standard would be used throughout.

In addition to the samples furnished, the collaborators were asked to determine carotene and chlorophyll in at least one sample of fresh, green plant tissue of their own choice by each of the specified methods, to test agreement of results from the different technics with such material.

Since the use of visual colorimeters for evaluating pigment concentrations is simple and quite well standardized, and moreover is being supplanted rapidly by photoelectric colorimeters and spectrophotometers, collaborators in this study were asked to confine themselves to the use of these latter instruments, to furnish results from both kinds of instruments if available, to state the types and makes employed, and to give a brief description of the light filters used in the photoelectric colorimeters for the different pigments. Those having spectrophotometers were requested to determine an extinction coefficient at 6650Å for the chlorophyll standard dissolved in 85 per cent acetone.

The collaborators were also requested to comment on the relative advantages or disadvantages of the different methods and to make suggestions for future collaborative work.



The results obtained by collaborators with the procedures used on the samples furnished them and on fresh samples of their own selection are given in Tables 1, 2, and 3.

TABLE 1.—*Carotene*<sup>1</sup> in collaborative samples of dry plant tissue  
Av. of duplicate determinations (mg. of carotene/100 grams of sample)

COLLABORATOR	EVALUATED BY—	ALFALFA MEAL					DRIED SPINACH LEAVES					DATE ANALYZED 1940
		PROCEDURES USED—					PROCEDURES USED—					
		PETERSON- HUGHES- FREEMAN	PETERING- WOLMAN- HIBBARD	MODIFIED PETERING- WOLMAN- HIBBARD	OTHERS		PETERSON- HUGHES- FREEMAN	PETERING- WOLMAN- HIBBARD	MODIFIED PETERING- WOLMAN- HIBBARD	OTHERS		
2	Spectrophotometers	6.07	4.89	5.87	5.78 <sup>2</sup>		13.90	11.40	13.30	12.78 <sup>3</sup>	7/12-18	
3	Bausch & Lomb, Visual	7.83	—	—	5.13 <sup>4</sup>		12.08	—	—	13.90 <sup>4</sup>	9/30-10/5	
4	Cenco-Sheard Spectrophotometer	7.09	—	8.52	—		15.20	—	17.45	—	9/25	
5	Bausch & Lomb, Visual	6.00	5.70	6.12	—		12.85	12.34	12.82	—	7/25	
6	Bausch & Lomb, Visual	7.35	8.15	6.40	—		15.55	17.60	17.40	—	7/17-19	
9	Cenco-Sheard Spectrophotometer	5.81	5.80	5.66	—		14.10	13.65	14.00	—	7/5 - 9	
	Av.	6.36	6.14	6.51	—		13.96	13.75	14.93	—		
	Max.	7.35	8.15	8.52	—		15.55	17.60	17.45	—		
	Min.	5.81	4.89	5.67	—		12.08	11.40	13.30	—		
	Range	1.54	3.26	2.65	—		3.57	6.20	4.15	—		
	Photoelectric Colorimeters											
1	Make Klett-Summerson with log scale	7.40	8.65	10.80	—		10.75	13.90	14.90	—	9/23-10/4	
2	KWSZ Trans. Range 4000-4650 Å. A blue + yellow glass 1% CuSO <sub>4</sub> solution	5.80	5.07	5.95	—		13.20	11.20	12.90	—	7/12-18	
		5.19 <sup>4</sup>	—	4.55 <sup>4</sup>	—		12.45 <sup>4</sup>	—	11.96 <sup>4</sup>	—		
3	KWSZ Corning No. 554	5.80	—	—	6.55 <sup>2</sup>		12.15	—	—	13.95 <sup>3</sup>	9/30-10/5	
4	Evelyn No. 440 Max. trans. at 4400 Å	7.40	—	9.80	5.00 <sup>4</sup>		13.20	—	18.50	13.50 <sup>4</sup>	9/25	
5	KWSZ Waco No. 2	6.05	5.71	5.95	—		12.95	12.12	12.40	—	7/25	
6	Evelyn Max. trans. 4600 Å	7.25	8.50	6.00	—		15.00	17.30	17.45	—	7/17-19	
7	Evelyn limits 4100-4750 Å	5.40	3.80	5.80	6.20 <sup>4</sup>		10.05	8.25	10.45	10.60 <sup>4</sup>	10/2- 9	
9	Cenco-Sheard Corning No. 554	6.20	6.50	6.60	—		15.30	15.25	15.95	—	7/5 - 9	
	Max. trans. at approx. 4400 Å											
	Av.	6.41	6.37	7.27	—		12.83	13.00	14.65	—		
	Max.	7.40	8.65	10.80	—		15.00	17.30	18.50	—		
	Min.	5.40	3.80	5.80	—		10.05	8.25	10.45	—		
	Range	2.00	4.85	5.00	—		4.95	9.05	8.05	—		

<sup>1</sup> Throughout this report the yellow pigment evaluated in petroleum benzin extracts is referred to as carotene.

<sup>2</sup> Russell, Taylor, and Chichester, *Plant Physiol.*, 10, 325 (1935).

<sup>3</sup> Obtained after passing the petroleum benzin extract through a column of CaHPO<sub>4</sub> · 2H<sub>2</sub>O. See No. 4 under Comments of Collaborators.

<sup>4</sup> Obtained after treatment with MgCO<sub>3</sub> according to the technic of Fraps, Kemmerer, and Greenberg. See references in review of literature, and also No. 2 under Comments of Collaborators.

<sup>5</sup> Obtained by the diacetone method. See No. 7 under Comments of Collaborators.

TABLE 2.—*Carotene in fresh tissue of optional choice*  
*Av. of duplicate determinations (mg./100 grams of sample)*

COLLABO- RATOR	TISSUE ANALYZED	PROCEDURES USED—			
		PETERSON-HUGHES- FREEMAN	PETERING-WOLMAN- HIBBARD	MODIFIED PETERING- WOLMAN-HIBBARD	OTHERS
Spectrophotometers as designated in Table 1					
2	Turnip greens	5.07	2.88	2.66	—
4	Rye leaves	9.80	—	10.70	9.50 <sup>1</sup>
5	Oat leaves	11.75	10.81	11.18	—
6	Soybean leaves	20.75	28.50	18.35	—
9	Soybean leaves	16.18	15.85	15.04	—
Photoelectric colorimeters and light filters as designated in Table 1					
1	Spinach	—	5.35	6.10	—
2	Turnip greens	4.60	2.88	2.65	—
4	Rye leaves	9.40	—	10.75	9.40 <sup>1</sup>
5	Oat leaves	11.66	10.75	11.12	—
6	Soybean leaves	20.20	29.40	18.30	—
7	Corn leaves	15.05	12.25	14.80	18.30 <sup>2</sup>
9	Soybean leaves	17.04	17.28	16.48	—

<sup>1</sup> Obtained after passing the petroleum benzin extract through a column of dicalcium phosphate. See No. 4 under Comments of Collaborators

<sup>2</sup> Diacetone method. See No. 7 under Comments of Collaborators.

TABLE 3.—*Chlorophyll values obtained by collaborators with the*  
*Petering-Wolman-Hibbard method and others as designated*  
*Av. of duplicate determinations (mg./100 grams of sample)*

COLLABORATOR	EXTINCTION <sup>1</sup> COEFFICIENT	COLLABORATIVE SAMPLES		FRESH TISSUE (OPTIONAL CHOICE)
		ALFALFA MEAL	DRIED SPINACH LEAVES	
Spectrophotometers as designated in Table 1				
2	30.0	751.6	1043.0	Turnip greens 15.1
6	32.3	722.0	1142.0	Soybean leaves 916.0
9	30.5	738.0	1020.0	Soybean leaves 689.7
	Av.	737.2	1068.3	
	Range	29.6	122.0	
Photoelectric colorimeters as designated in Table 1				
Light Filters Used				
6	Evelyn No. 660 Trans. limits 6350-7200 Å	735.0	1015.0	Soybean leaves 876.0
7	Corning Nos. 243 & 397	534.0 539.0 <sup>2</sup>	760.0 811.0 <sup>2</sup>	Corn leaves 459.0 538.0 <sup>2</sup>
9	Corning Nos. 243 & 396. Max. trans. above 6200 Å	716.0	1033.0	Soybean leaves 667.0
	Av.	661.7	936.0	
	Range	201.0	273.0	

<sup>1</sup> Determined for 5X chlorophyll in 85% acetone at 6650 Å.

<sup>2</sup> Obtained by extraction with 95% ethanol. See No. 7 under Comments of Collaborators.

## COMMENTS OF COLLABORATORS

A. *Carotene*.—1. I have added no comments to the data sheets because we have been so hurried that it was impossible to check back on the difficulties we encountered to make sure whether they were inherent in the method or matters of technic. The recommended method for aldehyde-free alcohol<sup>11</sup> was most unsatisfactory as we carried it out. Alcohol prepared by the m. phenylenediamine method and saturated with KOH immediately before use gave blanks that were negative in the colorimeter.

2. The Peterson-Hughes-Freeman method has the following advantages over the other two methods: (a) It is more rapid. (b) There is much less danger of destruction of carotene. The carotene content of Sample 3 (fresh turnip greens) was found to be much higher when the Peterson-Hughes-Freeman method was used than when either of the other methods was employed. Grinding with sand as directed in the Petering-Wolman-Hibbard procedures has been shown in our laboratory and others to destroy considerable carotene in many fresh green materials. This destruction is not so great in the dried samples. (c) It extracts less impurities that absorb light in the same region of the spectrum. The petroleum benzin extracts from Samples 1 and 2 obtained by the Peterson-Hughes-Freeman method and the modified Petering-Wolman-Hibbard procedure were treated with a specially activated  $MgCO_3$  developed in our laboratory to remove impurities from carotene solutions with the following results (No. 2, Table 1).

The modified Petering-Wolman-Hibbard procedure has the advantage over the original procedure of being somewhat more rapid. Neither the Petering-Wolman-Hibbard procedure nor the modified method is as reliable as the Peterson-Hughes-Freeman procedure.

The Peterson-Hughes-Freeman method submitted should be modified to correspond to the method tentatively adopted by the A.O.A.C. The extraction with 85% methanol is not needed; 90% methanol should be used throughout. For fresh, green materials this collaborator used the procedure suggested by Peterson and his associates.<sup>12</sup>

4. From the time the samples were received until they were run they were stored at  $-20^{\circ}C$ . The high results obtained with the modified Petering-Wolman-Hibbard procedure may be due in part to the presence of chlorophyll in the final extract since no corrections were made for this pigment. Since this collaborator did not work with chlorophyll, the original Petering-Wolman-Hibbard method was not used.

The other method used consisted of pouring the petroleum benzin extracts through a column of dicalcium phosphate. A paper describing the details has been published.<sup>14</sup> It will be noted that much lower results were obtained by the latter method than by the others used. The dicalcium phosphate supposedly removes all the non-carotene chromogens. When the extracts from the Peterson-Hughes-Freeman and Petering-Wolman-Hibbard methods were passed through columns of dicalcium phosphate the non-carotene chromogens were removed and the values obtained agreed more consistently. This raises the question as to the accuracy of any method utilizing the Willstätter-Stoll principle, especially where the materials have been exposed to air for some time or to the action of the digestive tract. Apparently 90% methyl alcohol does not remove all these non-carotene chromogens, so that they are determined as carotene.

5. My comments may be taken from Abstracts of Papers, 100th Meeting of the American Chemical Society, Detroit, Mich., Sept. 1940, Division of Biological Chemistry, p. 12.

The Petering-Wolman-Hibbard method, which utilizes barium hydroxide for the

<sup>11</sup> *Ind. Eng. Chem., Anal. Ed.*, 5, 100 (1933).

<sup>12</sup> *Kansas Agr. Exp. Sta. Tech. Bull.* 46, p. 21 (1940).

removal of chlorophyll shows considerable promise for the determination of carotene in dry or fresh plant tissue. This method has been compared with the Peterson-Hughes-Freeman procedure. The two methods give comparable results on a variety of plant tissues, provided certain conditions in the barium hydroxide technic are carefully observed. The sample or aliquot must be sufficiently small so that the quantity of barium hydroxide recommended will effectively remove all the chlorophyll. Best results have been obtained by the use of a highly active carbonate-free material produced by filtering 15 ml. of hot, freshly prepared, 15% aqueous solution of  $\text{Ba}(\text{OH})_2$  octahydrate into 85 ml. of the acetone pigment extract to be digested. This obviates the necessity of starting with a carbonate-free  $\text{Ba}(\text{OH})_2$ . Differences between the results obtained by the two methods average about 5%. This variance has been found to be due almost entirely to the difficulty of removing 100 per cent of the carotene by grinding plant tissues with quartz sand under acetone as specified in the Petering-Wolman-Hibbard procedure. Well-extracted and apparently pigment-free residues from these grindings have been found to contain 2-13% of the original carotene when further saponified with alcoholic potash. Acetone-barium hydroxide digestion of this residue apparently is not so effective as alcoholic potash digestion.

The Peterson-Hughes-Freeman procedure when applied directly to fresh plant tissue (oat leaves) removes only 80% of the carotene. Alternate grinding of the digested residue under alcohol and petroleum removes another 10%. Redigestion of this ground residue in alcoholic potash accomplishes complete removal of the remainder. Some promising features of the barium hydroxide method are: (1) Xanthophylls are more readily removed with the petroleum from aqueous acetone solution than from aqueous alcoholic potash, and the simultaneous and complete removal of carotene is thus assured; and (2) the petroleum fraction of plant materials containing cryptoxanthin would probably give higher values since petroleum does not completely remove this pigment from alcoholic potash solutions. May I suggest that in the next study all extract residues be reground with alcoholic potash and digested, and their carotene content be reported separately so that the efficacy of extraction by grinding can be tested.

Most laboratories that have spectrophotometers in addition to photoelectric colorimeters, standardize the latter from time to time by means of the former; thus one should expect no large deviation in results from the two instruments from a given laboratory. In routine work greater reproducibility is certainly obtained by means of photoelectric colorimeters.

6. The Petering-Wolman-Hibbard method was compared only with the Peterson-Hughes-Freeman method. The method used in this laboratory for routine carotene determinations\* is not greatly different from the method of Peterson *et al.*, and has been shown to give results that are in agreement with results obtained by the Peterson-Hughes-Freeman method. Consequently we have used only the Peterson-Hughes-Freeman method in checking the Petering-Wolman-Hibbard method.

It was impossible to run all the samples in duplicate by all the methods in one day. To avoid having the solutions stand 2-3 days while the analyses were being completed, only one method was carried out on the two dry samples daily. These samples were stored at 3° C. in the dark and were removed only for analysis. The modified Petering-Wolman-Hibbard method for the determination of carotene showed no advantages over the Peterson-Hughes-Freeman method in speed or ease of manipulation. It is impossible to make any definite statement regarding the reliability of the various methods from such a limited number of analyses, but it appears that the Petering-Wolman-Hibbard method gives higher results than does either the modified procedure for carotene alone or the Peterson-Hughes-Freeman method, and this was especially evident in the fresh samples that we analyzed. We cannot

say whether these higher values are attributable to carotene or some other pigment.

The Peterson-Hughes-Freeman method has been generally accepted. If the results from this collaboration show that the Petering-Wolman-Hibbard method does not agree with the Peterson-Hughes-Freeman method, further collaboration would be of no value until the causes for such discrepancies have been determined and a reliable method has been developed through individual researches. It appears that the Petering-Wolman-Hibbard method should have been compared with the Peterson-Hughes-Freeman method before this collaborative study was undertaken.

7. *Diacetone method.*—The sample was covered with 95% ethanol and refluxed 1 hour, and the alcohol was replaced by fresh solvent. This extraction was repeated until the last alcohol solution was colorless (usually 3 times). The volume of the combined extract was noted, and a 25 ml. aliquot was removed. To this was added 50 ml. of water containing 10% of NaCl and 1% of  $\text{Na}_2\text{CO}_3$ , and the solution was extracted with Skellysolve "B" until the latter became colorless (ca. 50 ml. total). To the Skellysolve solution was added 15 ml. of 10% KOH in methanol, and the mixture was placed overnight in the refrigerator. The next day 30 ml. of water was added, and the mixture was shaken out with Skellysolve until the latter became colorless. The Skellysolve solution was washed once with 15 ml. of water, and then shaken out with 15 ml. portions of diacetone alcohol until the last portion remained colorless (usually 3 times). The Skellysolve was again washed with 15 ml. of water, diluted to 100 ml. (or other convenient volume), and the carotene content was determined by reading on the Evelyn colorimeter, and referring to a standard curve prepared with a solution of beta carotene in Skellysolve "B."<sup>10</sup> The extraction of the plant pigments by grinding with 85% acetone seems unsatisfactory. Our experience has indicated that carotene is lost very rapidly during the maceration of fresh, green, plant tissues unless some method of preventing enzymatic oxidation is used. In the Petering-Wolman-Hibbard procedure there is an appreciable interval during which the sample is macerated and no solvent is present. It is possible that destruction during this time may partially account for the lower results obtained on the fresh sample by this method in comparison with the diacetone method.

Furthermore, extraction by grinding with the solvent is very tedious, requiring between 1 and 2 hours per sample. This feature renders the method impracticable for application to a large number of samples, and may result in varying completeness of extraction according to the energy and enthusiasm of the analyst. The main limiting factor in analyzing a large series of samples for carotene is the time required. Any shortening of the methods would be a decided improvement. In all three of the proposed carotene methods difficulty was experienced in extracting xanthophyl from the petroleum benzin solution by shaking out with 85 or 90% methanol; 6-7 extractions were required in each case. In contrast, diacetone alcohol or 85% ethanol appears to be more effective for this purpose. An inherent difficulty in all of the carotene methods lies in this phasic separation of carotene and xanthophyl. It is probable that the separation is never absolutely sharp, and that errors are thereby introduced. Perhaps a detailed study of the solubility and distribution of pure carotenoids in various solvents would improve this situation. In the light of the results herewith reported it appears that more collaborative work is needed, especially with respect to fresh, green material.

8. (Although unforeseen circumstances prevented this collaborator from submitting results to be included in the report, he was kind enough to raise certain questions in the light of his own experience that appeared to be of sufficient general interest to include here.)

In reading the method as published by Petering, Wolman, and Hibbard, a few points occur to me, which may need further attention. The most important thing I believe, is that the size of sample, namely 1 or 2 grams of fresh tissue, which is

taken for analysis, is so small that it would be utterly impossible to obtain reliable results without running a large number of samples and taking the average of the values. We have analyzed a large number of samples of fresh crops in the course of our work on silages and find that even with a 25 gram sample the difference of the 2 duplicate determinations may run 100% or over and we ascribe this difference largely to sampling difficulties. Another possible source of error is the length of time that may elapse during the preliminary cutting of the sample for analysis. I believe that the carotene content of fresh tissue may change fairly rapidly under such conditions so that a long period of cutting in order to get better sampling will cause a larger amount of destruction of carotene. The same difficulty, namely, the destruction of carotene, may occur during the maceration processes unless care is taken to keep the sample covered with acetone at all times.

9. It is quite generally agreed that the Peterson-Hughes-Freeman method for determining carotene possesses advantages over many earlier procedures in ease of manipulation and rapidity. Some of the modifications proposed in the tentative A.O.A.C. method; e.g., use of only 90% methanol for removing xanthophyl and permitting this reagent to remove part of the residual alkali, thereby reducing the number of washings with water, augment these advantages. The second period of digestion, following grinding of the residue from the primary digestion, recommended for samples of fresh plant tissue, increases the time required for analysis of such materials. Soluble alkali in the presence of ethanol is thought by some investigators to cause with certain tissues the formation and precipitation of substances that interfere with the complete extraction of carotene.

In the original and modified Petering-Wolman-Hibbard procedures the use of barium hydroxide, instead of the conventional soluble alkali, for removing chlorophyl from extracts of plant tissue is unique and effective for this purpose, and eliminates the necessity for removing residual soluble alkali. The acetone used as the extractant is pleasanter to handle than ethanolic alkali, eliminates the necessity for preparing aldehyde-free ethanol, and permits easier transfer of residual tissue from digestion flasks to funnels. In considering the time required by these procedures, the analyst should remember that the extract obtained by the original procedure permits evaluation of both carotene and chlorophyl in a much shorter time than was required to achieve the same result by earlier procedures. The modified procedure requires less time than the original for determining carotene, but is designed for this pigment only. In routine work the collaborators have found it to be shorter than the Peterson-Hughes-Freeman method, especially with fresh tissue, which it was thought necessary to redigest when the latter method was employed. Several different assistants in the Associate Referee's laboratory acquired the technic easily and had no difficulty in obtaining consistent results with the Petering-Wolman-Hibbard procedures. When photoelectric colorimeters are used to evaluate carotene concentrations by these procedures, it is advisable to take readings with light filters that will permit making the chlorophyl correction, which may be required in individual cases.

If careful technic is observed the collaborators have found these procedures to give carotene values in close agreement with those obtained by the Peterson-Hughes-Freeman technic for the majority of tissues analyzed. It is believed that investigators desiring to determine both pigments may use the original Petering-Wolman-Hibbard method, and if careful attention is paid to technic, safely feel that for most tissues the carotene values obtained are in line with those which the Peterson-Hughes-Freeman procedures would give. If carotene alone is to be determined the use of the Peterson-Hughes-Freeman method or the modified Petering-Wolman-Hibbard procedure might be left to personal choice. If complete extraction of carotene from the tissue by each of these procedures is assumed, there appears

to be no fundamental reason why values obtained by their use should not agree, since they all use the same means of isolating carotene. Some investigators criticize the phasic separation of xanthophyl from carotene as practised by these procedures for not removing all non-carotene impurities from the petroleum benzin, especially when old plant materials that have been exposed to the air for a long time are being analyzed. Should further investigation show other means for isolating carotene to be definitely superior, these technics should be utilized where necessary.

*B. Chlorophyl.*—2. The purity of the chlorophyl standard is questionable. There seemed to be a slight amount of uncolored material, which was very difficult to dissolve. I suggest that in future work the extinction coefficient on a number of chlorophyl preparations be determined so as to set up a standard for purity.

6. The Petering-Wolman-Hibbard method was the only method used in the determination of chlorophyl. We therefore decline to comment upon the relative merits of the method.

7. *Other method used (see also Table 3).*—The sample was covered with 95% ethanol refluxed 1 hour, and the alcohol was replaced by fresh solvent. This extraction was repeated until the last alcohol solution was colorless (usually 3 times). The volume of the combined extract was noted, and the chlorophyl content was determined by reading on the Evelyn colorimeter and referring to a standard curve prepared with a solution of 5X chlorophyl in 95% ethanol.

It will be noted that our procedure for chlorophyl is identical with that of Petering-Wolman-Hibbard, except that boiling 95% alcohol is used for the extraction in place of grinding with 85% acetone. The latter extraction procedure has been discussed in connection with the carotene methods. The direct photoelectric determination of the chlorophyl content of the extract is extremely convenient, and has been used on a large number of corn samples in this laboratory.

9. Most earlier methods for determining total chlorophyl involved its extraction from plant tissue with acetone or alcohol, removal from the extractant with diethyl ether, saponification therein with alcoholic alkali, and colorimetric or spectrophotometric evaluation of the saponification products after their separation with water. The evaluation of total chlorophyl directly in the extract of the tissue, as practised in the Petering-Wolman-Hibbard method, eliminates these intermediate steps and greatly facilitates determination of this constituent. Since this method depends upon essentially the same means for removing the pigment from the tissue, there appears to be no fundamental reason why values so obtained should not agree with those obtained by the earlier procedures, and errors due to manipulative losses should be appreciably reduced. Hand-grinding with sand and cold extractant to effect complete extraction of pigments is tedious, but is perhaps inevitable because of the nature of plant tissue and of the constituents being determined. This is especially true with fresh tissue, which cannot be reduced mechanically, or with any material of which a comparatively large amount must be used to insure a representative sample. A machine that would thoroughly disintegrate plant tissue under an appropriate extractant without loss, thereby eliminating hand grinding and insuring rapid inactivation of enzymes, exclusion of air from the tissue during its disintegration, and complete extraction of pigments, would be an invaluable adjunct to this procedure.

The Associate Referee and his assistants experienced no difficulty in relating light transmission and concentration of the 5X chlorophyl in 85 per cent acetone by means of a photoelectric calorimeter as directed in the method, but did have difficulty in obtaining consistent values for an extinction coefficient for this material with the spectrophotometer. The average value given in Table 3 is the result of many determinations.

## DISCUSSION

Since the procedures used in this study appear elsewhere in *This Journal*, or in others easily accessible, it seems unnecessary to reproduce them in detail.

Table 1 shows wide variations in carotene values from different collaborators with all methods used on the dried samples, although the average values obtained by the three methods each was asked to use agree fairly well. No method appears to give consistently higher or lower results. The range of variation in results from different collaborators for the Petering-Wolman-Hibbard procedures is somewhat greater than that for the Peterson-Hughes-Freeman method. This is possibly due to less experience among the collaborators with the barium hydroxide technic than with methods involving digestion with alcoholic alkali and failure on the part of some to apply the chlorophyll correction recommended for the former procedures when necessary. The date of analysis seems to have had no consistent effect in determining whether the results from a given collaborator are low or high. Agreement between the average values obtained by photoelectric colorimeters and spectrophotometers is good. This was to be expected, but is encouraging, since the former instruments are certainly better adapted for routine evaluation of carotene than the latter.

Carotene values determined by the two adsorption technics represented are lower than those obtained by the same investigator with the methods depending upon the Willstätter-Stoll phasic separation. It is suggested by the collaborators obtaining them that these lower values represent more nearly pure carotene than do the others, because of more effective removal of pigment impurities. It may be necessary to decide by bioassays which values most nearly represent the biologically active carotenoids. It might be pointed out that the other methods could readily be adapted to another means of removing xanthophyll, in case one is shown to be definitely superior, and could be used conveniently and with assurance in routine work.

Values by the diacetone method are higher than those obtained with the other methods by the same investigator. Excellent agreement between values from the Peterson-Hughes-Freeman and the Russell-Taylor-Chichester methods were obtained by the collaborator using them.

The results in Table 2 indicate no superiority or inferiority of any method used for determining carotene in fresh plant tissue. One of the collaborators obtained radically lower results with the Petering-Wolman-Hibbard procedures as compared to those obtained by the Peterson-Hughes-Freeman technic; one got definitely higher results with the original Petering-Wolman-Hibbard procedure; whereas, in the hands of the others the different technics gave values in fairly good agreement for the tissues analyzed. The use of dicalcium phosphate gave values in excellent agreement with those by the Peterson-Hughes-Freeman technic and in



fairly good agreement with those by the modified Petering-Wolman-Hibbard procedure for carotene in rye leaves. The diacetone method gave a value for carotene in corn leaves appreciably higher than the values obtained by the other methods.

It should be mentioned that the Associate Referee neglected to point out to the collaborators that in the original Petering-Wolman-Hibbard procedure grinding once or twice with 100 per cent acetone, and later adding water to bring it to the proper dilution before the barium hydroxide treatment, facilitate complete extraction of the pigments from the tissue. It is possible that the lower carotene values obtained by some of the collaborators with this technic could have been brought into closer agreement with the others by this means.

Although the number of collaborators represented in Table 3 is small, agreement among values for total chlorophyll in the collaborative samples by the Petering-Wolman-Hibbard method is exceptionally good in three out of four cases. This is encouraging, because a need is felt among plant investigators, concerned with studies of chlorosis or other disorders that affect pigment formation, for a convenient, practicable method for evaluating total chlorophyll. Since the use of the photoelectric colorimeter with this method reduces the time and work required for the determination of this pigment, it appears that the Petering-Wolman-Hibbard technic should help to fill this need.

The values obtained by extracting with boiling 95 per cent ethanol agree fairly well with those the same collaborator obtained with the Petering-Wolman-Hibbard procedure for the collaborative samples, but all are radically lower than those determined by the other collaborators. No comparisons are possible among results from the fresh tissues analyzed, since all are different samples, except that extraction with boiling 95 per cent alcohol gave a value for chlorophyll in corn leaves somewhat higher than that obtained by the acetone extraction.

The extinction coefficients determined for the 5X chlorophyll in 85 per cent acetone by the three collaborators represented are of the same order. However, Mackinney,<sup>18</sup> Zscheille, private communication, and others have pointed out the difficulty of evaluating an extinction coefficient for use in the determination of this pigment, because of the variation in the proportion of a and b chlorophyll in the preparations available for purchase and in extracts in which total chlorophyll might be evaluated. Hence, it would be a mistake for anyone to use the constants given here, without satisfying himself that the primary standard he is using gives similar values.

The results obtained in this study make it appear that further collaborative effort toward unification of methods available for determining both carotene and total chlorophyll would be profitable.

## RECOMMENDATIONS\*

It is recommended—

(1) That the study of methods for the determination of carotene and chlorophyl in plant tissue be continued, and that as many as possible of the promising procedures available for this purpose be given consideration.

(2) That the Petering-Wolman-Hibbard procedure be given additional study as a combined method for determining total chlorophyl and carotene, and that in the next collaborative work increased attention be directed toward evaluation of chlorophyl.

(3) That a study be made to determine the best methods for sampling fresh plant tissues and for handling samples of such materials prior to and during extraction of pigments therefrom for quantitative evaluation.

(4) That a study be made of the possibility of using a machine in these procedures to disintegrate samples of plant tissue under an appropriate extractant, thereby eliminating hand grinding.

## COLLABORATORS

To these men, who cooperated to make this report possible, the Associate Referee expresses his sincere appreciation:

(1) M. A. Freeman, Dept. of Chemistry, State College, Amherst, Mass.

(2) A. R. Kemmerer, Div. of Chemistry, Agr. Exp. Station, College Station, Tex.

(3) W. E. Krauss, R. G. Washburn, and J. D. Sayre, Dept. of Dairy Industry, Agr. Exp. Station, Wooster, Ohio.

(4) L. A. Moore, Dept. of Dairy Industry, Agr. Exp. Station, East Lansing, Mich.

(5) W. J. Peterson, Dept. of Chemistry, State College, Manhattan, Kan.

(6) W. C. Sherman and C. J. Koehn, School of Agr., Alabama Polytechnic Inst., Auburn, Ala.

(7) F. M. Strong and John Porter, Dept. of Biochemistry, College of Agr., Univ. of Wisconsin, Madison, Wis.

(8) M. W. Taylor, Dept. of Biochemistry, Agr. Exp. Station, New Brunswick, N. J.

(9) Myron Seeder (in cooperation with the Associate Referee), Agr. Exp. Station, East Lansing, Mich.

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No report on hydrocyanic acid was given by the associate referee.

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No report on lignin was given by the referee.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 48 (1941).

## REPORT ON ENZYMES (PAPAIN)

By R. F. THOMPSON (Agricultural Experiment Station,  
Honolulu, Hawaii), *Referee*

It has been observed that in the preparation of samples of papain for assay by the methods of Balls *et al.*, *This Journal*, 18, 140 (1935); *J. Biol. Chem.*, 121, 737 (1937), the time factor is important. The method calls for grinding the sample in a mortar with a little water and then making it up to volume. In this method and the one described in the following paragraph, the enzyme solution must be assayed immediately, as the activity decreases rapidly upon standing.

The method suggested, which has been used by the Referee in more than a thousand assays, consists of placing weighed samples of papain in glass-stoppered, graduated cylinders and adding water to the correct dilution (i.e., 50 mg. diluted to 25 ml.) and shaking for a period of 5 minutes. Samples of the enzyme solution are then withdrawn and assayed at once. To show the effect of over-shaking or standing in solution, several samples were shaken for a long period of time, and portions were withdrawn at short intervals and assayed. The following table illustrates the decrease in activity:

Time of shaking* <i>minutes</i>	Time of clotting (seconds)		
	Sample 7	Sample 8a	Sample 9a
3	37	33	34
5	35	32	40
7	37	—	—
10	41	35	44
15	—	38	—
20	54	42	49
30	—	57	—

\* Shaking was done in a mechanical shaker at about 300 shakes per minute.

From these results it is logical to deduce that too much shaking or allowing the sample to stand too long is detrimental to the activity in the papain, and that in preparing samples by either method, the assay should be made as soon as possible after the latex has dissolved in water, preferably at the 5-minute interval.

## REPORT ON WATER, BRINE, AND SALT

## FLUORINE IN WATER

By A. E. MIX (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

As a result of the Referee's recommendation in last year's report, *This Journal*, 23, 447 (1940), the thorium nitrate titration method for the determination of fluorine was adopted as tentative. It was published in *Methods of Analysis*, A.O.A.C., 1940, p. 529.

TABLE 1.—*Collaborative results*

COLLABORATOR	FLUORINE FOUND	AVERAGE	AV. DEV.
	p.p.m.		
	With hydroxylamine hydrochloride		
1	1.02		
	1.04		
	1.05	1.05	0.023
	1.10		
	1.07		
	1.04		
3	.91	.94	.03
	.97		
4	1.00		
	0.95	.98	.023
	1.00		
5	1.17	1.17	.00
	1.17		
6	1.14	1.13	.01
	1.12		
7	1.03	1.035	.005
	1.04		
8	1.025	1.000	.025
	.975		
	Without hydroxylamine hydrochloride		
1	1.02		
	1.04		
	1.11	1.06	0.033
	1.02		
	1.07		
	1.10		
2	1.00		
	0.88	0.88	.083
	0.75		
3	.91	.94	.03
	.97		
4	1.20		
	0.98	1.07	.083
	1.04		
5	1.15	1.16	.01
	1.17		
6	1.13		
	1.15	1.15	.008
	1.15		
	1.16		
7	1.03	1.035	.005
	1.04		
8	1.075	1.050	.025
	1.025		

The Referee found later that this method required certain changes. Therefore a sample of artificial mineral water containing 2193 p.p.m. of chlorides, 1465 p.p.m. of sulfates, 28 p.p.m. of phosphates, and 1.00 p.p.m. of fluorine was sent to the collaborators for examination according to these changes, which will be discussed later in this report.

The collaborators' results are summarized in Tables 1 and 2.

TABLE 2.—*Comparison of collaborative results*

	AVERAGE RECOVERY	ERROR	NO. OF TRIALS	STANDARD DEV.
		<i>per cent</i>		
A	1.043	4.3	19	.071
B	1.049	4.9	24	.101
C	1.046	4.6	43	.076

F (present) = 1.00 p.p.m.

A = With hydroxylamine hydrochloride.

B = Without hydroxylamine hydrochloride.

C = Combined results.

Last year's collaborative study gave an error of 3.3 per cent for 41 trials and a standard deviation of 0.230 on a sample containing 2.11 p.p.m. of fluorine. This year's study gives for 43 trials an error of 4.6 per cent and a standard deviation of 0.076 on a sample containing 1.00 p.p.m. of fluorine. These results indicate that while the error has been increased 1.3 per cent, the precision is 3 times greater than that of last year. Therefore, the method with the suggested changes can be used to recover with accuracy and precision 1.00 p.p.m. of fluorine.

The changes submitted this year follow:

1. The addition of 1 ml. of 0.1% hydroxylamine hydrochloride to the aliquot titrated.
2. The use of a standard color comparison tube.
3. The necessity for running a blank for each distillation of fluorine.
4. Evaporation of the sample before distillation in porcelain or platinum over a Bunsen flame just below the boiling point.
5. Preliminary determination of chlorides and their precipitation in the distilling flask with silver perchlorate.

1. When an aliquot of the distillate is titrated with thorium nitrate in the presence of alizarin red, a colloidal haze is formed at the pink end point, which prevents an accurate color comparison of the sample tube and the standard tube. The addition of 1 ml. of 0.1 per cent hydroxylamine hydrochloride to each tube will dispel the haze and give a transparent pink. A comparison of Columns A and B of Table 2 shows that the use of hydroxylamine hydrochloride is a distinct improvement.

2. As an aid in color comparisons of the sample and standard tubes, a standard color comparison tube was used. This tube contains 40 ml. of

distilled water, 2 ml. of 0.05 *N* hydrochloric acid, 1 ml. of alizarin red indicator solution, 1 ml. of 0.1 per cent hydroxylamine hydrochloride, and sufficient thorium nitrate to give a faint but definitely pink end point. All other end-point colors should be compared with the one in this tube.

3. It is necessary that a blank determination be made for every distillation of a sample. In her own analyses the Referee found very few blanks to contain 0.00 p.p.m. of fluorine. Should the analyst fail to subtract a blank from a sample containing a small concentration of fluorine, he would by necessity introduce a serious error. For example, the blanks reported by the collaborators showed from 0.04 to 0.15 p.p.m. of fluorine, which if not subtracted from the sample (1.00 p.p.m. of fluorine) would introduce an error of 4–15 per cent.

4. Slow evaporation of the sample in glass or in porcelain will also introduce an appreciable error. The sample should be kept alkaline to phenolphthalein and quickly evaporated to 20 ml. in porcelain or platinum (not glass) over a Bunsen flame just below the boiling point. Blanks evaporated in this manner were found to give lower concentrations of fluorine than did blanks evaporated slowly over steam by the old method.

5. Chlorides should be determined by titration with standard silver nitrate in the presence of potassium chromate. An equivalent amount of silver perchlorate should then be added directly to the distilling flask to precipitate the chlorides. Failure to observe this step will produce excess acidity in the distillate. Since the sample sent out this year was high in chlorides, a heavy precipitate remained in the bottom of the flask after the silver perchlorate was added. This caused considerable bumping. Anticipating a suggestion from the collaborators that the silver chloride should be filtered off before distillation, the Referee made three such determinations. An average recovery of 0.71 p.p.m. of fluorine was obtained on a sample containing 1.00 p.p.m. of fluorine. Apparently filtering off the silver chloride withholds the fluorine ion and produces incomplete recovery. This method of removing chlorides prior to distillation should be further investigated. The difficulty of bumping due to excess silver chloride in the distilling flask will only be encountered occasionally since most mineral waters contain less chlorides than did the sample sent out this year.

The Referee established a lower limit of the concentration of fluorine that can successfully be determined by the thorium nitrate method at 0.1 p.p.m. A sample containing 0.1 p.p.m. of fluorine was distilled and analyzed. Seven trials gave 0.09, 0.10, 0.10, 0.15, 0.10, 0.15, 0.10 p.p.m. of fluorine, an average of 0.113 p.p.m., or an error of 13 per cent. Although the method is sensitive enough to determine 0.1 p.p.m. of fluorine, the Referee does not suggest its use for such small quantities. One drop of the standard sodium fluoride solution is equal to 0.05 ml., or 0.025 p.p.m. of fluorine. Should the analyst exceed the real end point by merely one

drop of this solution, he would introduce an error of 25 per cent for a sample containing as little as 0.1 p.p.m. of fluorine.

The Referee found this method to be especially applicable to solutions containing 1.00–3.00 p.p.m. of fluorine. In this range the expected error will be from 3.3 to 4.6 per cent and the expected standard deviation from 0.071 to 0.230.

The Referee expresses appreciation to the collaborators and to P. A. Clifford and M. Millman of the U. S. Food and Drug Administration for their assistance in this work.

It is recommended\* that the method presented last year with the changes suggested and discussed this year be adopted as official, first action.

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No report on moisture in effervescent salts was given by the referee.

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### REPORT ON DAIRY PRODUCTS

By G. G. FRARY (State Chemical Laboratory,  
Vermillion, S. Dak.), *Referee*

Several of the associate referees that were appointed for work this year chiefly from laboratories in the States, were not able to complete or give attention to the work. In the case of the Referee's laboratory, the assistant that was assigned to work on solids and ash resigned, so that work has not been completed. Attention should be given to the work of Sanders in the Bureau of Dairy Industry, on determination of chlorine in milk. He has shown that the official method results in loss of chlorine, and the importance of this is that determination of chlorine in milk is a valuable test for detecting certain diseases. Therefore, in considering revision of the official method, this matter should be taken into consideration.

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### REPORT ON BUTTER

By J. A. MATHEWS (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

At the last meeting of the Association, the stirrer method for the preparation of butter samples was adopted as tentative. Since then the method has been extensively used in the field laboratories of the Food and Drug Administration, and no difficulties have been reported. It is obviously impractical to study such a method collaboratively in the usual way. The submission of standard samples for preparation by the collaborators would involve previous mixing, which would to a large extent defeat the purpose of the experiment. If the collaborators were requested to furnish their own

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 82 (1941).

samples and report on that basis, results would be duplications of those obtained in developing the method and would certainly be of less value than the extended satisfactory experience with the method under practical working conditions.

It is therefore recommended\* that the stirrer method for the preparation of butter samples described in *Methods of Analysis, A.O.A.C.*, 1940, 293, 97-98, be adopted as official, first action, without further study.

Work has been started on the development of a rapid method for the direct determination of fat in butter, and it is recommended that the study be continued for another year.

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No report on cheese (isolation of fat) was given by the associate referee.

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No report on sampling of soft cheeses was given by the associate referee.

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No report on malted milk (fat) was given by the associate referee.

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## REPORT ON CASEIN IN MALTED MILK

By IMAN SCHURMAN (U. S. Food and Drug Administration,  
Cincinnati, Ohio), *Associate Referee*

At the last meeting of the Association the Committee on Recommendations recommended that the study of casein in malted milk be continued.

After a review of the methods commonly used for the determination of casein, the Associate Referee decided that the present A.O.A.C. method for milk proteins in milk chocolate, *Methods of Analysis, A.O.A.C.*, 1935, 198, might also be applicable for the determination of casein in malted milk.

For the application of the method to different brands of malted milk, two modifications were deemed necessary. Digestion of an aliquot of the sodium oxalate solution before and after precipitating the casein is extremely difficult owing to incessant foaming, *This Journal*, 22, 603 (1939). Some samples required from 2 to 3 hours of constant attention before the foaming ceased. In contrast, the digestion of the washed casein precipitate proceeds smoothly and requires practically no attention.

Trouble was also experienced in obtaining clear filtrates on certain samples. The addition of different quantities of acid and warming the solution before and after precipitation failed to yield clear filtrates. However, the addition of a small amount of paper pulp before precipitation and subsequent warming of the mixture to approximately 50° C. yielded clear filtrates.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 56 (1941).



After these ideas had been incorporated into the milk protein method, the following proposed modification was evolved:

#### CASEIN IN MALTED MILK AND CHOCOLATE MALTED MILK

Place a 10 gram sample in a 250 ml. (or larger) centrifuge bottle and extract with two 100 ml. portions of petroleum benzin by shaking until uniform, centrifuging, and decanting the supernatant layer. To the dry residue add exactly 200 ml. of 3%  $\text{Na}_2\text{C}_2\text{O}_4$  solution. Shake occasionally over a 4 hour period. Centrifuge for 15 minutes at high speed (1800 r.p.m. if No. 1 Sb bottle is used). Pipet 50 ml. (100 ml. for chocolate malted milk product) of supernatant liquid into a 250 ml. beaker. Add 50 ml. of paper pulp solution (1 filter paper) and 2 ml. of glacial acetic dropwise, with constant stirring. Set the beaker in warm water ( $45^\circ\text{--}50^\circ\text{C.}$ ) and let stand 15 minutes. Cool to room temperature and filter with moderate suction through a 70 cm. Büchner funnel previously fitted with No. 589 white ribbon paper and overlaid with a layer of paper pulp. Wash precipitate 2 or 3 times with cold water. (Filtrate should be clear, or nearly so.) If the first portions of the filtrate are not clear, repeat the filtration and complete the washing of the precipitate. Determine N in the washed precipitate and filter paper as directed in *Methods of Analysis*, A.O.A.C., 1940, 27, 25, and multiply by 6.38 to obtain the equivalent of casein. Correct result for blank on reagents and paper pulp.

Two authentic samples of malted milk were prepared in malted milk factories under the supervision of the Associate Referee. The casein content of each brand was calculated from the casein in the milk (determined by the tentative A.O.A.C. method for casein in milk, *Methods of Analysis*, A.O.A.C., 1940, 271, 13, and the total weight of the finished batch of malted milk. Per cent casein calculated: Brand 1, 4.82; Brand 2, 5.71.

In addition, laboratory mixed samples of chocolate malted milk were prepared from each brand. Each sample was prepared to contain 35 per cent malted milk, 50 per cent sugar, and 15 per cent cocoa.

Analysis of the authentic samples for casein by the proposed method gave the following results:

SAMPLE	MALTED MILK		CHOCOLATE MALTED MILK	
	FOUND	CALCULATED	FOUND	CALCULATED
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Brand 1	4.66	4.82	1.66	1.67
	4.72		1.60	
Brand 2	5.87	5.71	1.97	2.00
	5.74		2.00	
	5.68			
	5.87			

For the purpose of collaborative study, samples of Brand 2 and the proposed method were submitted to several collaborators. The results for casein are as follows:

COLLABORATOR*	MALTED MILK	CHOCOLATE MALTED MILK
	<i>per cent</i>	<i>per cent</i>
J. T. Field	5.71	2.02
St. Louis	5.64	2.00
J. P. Alden	6.05	2.09
Chicago	6.06	2.08
F. J. McNall	5.68	1.98
Cincinnati	5.74	1.98
M. L. Offutt	5.75	1.87
New York	5.64 Av. 5.59	1.99 Av. 1.93
	5.50	1.95
	5.47	1.90

\* All collaborators are members of the U. S. Food and Drug Administration.

#### COMMENTS BY COLLABORATORS

*J. T. Field.*—No particular difficulties were experienced with the method.

*F. J. McNall.*—The method works very well.

*Marie L. Offutt.*—The method works easily, but I believe the time could be cut down considerably from the four hours' extraction by the following procedure: "Add to the dry residue in the bottle with pipet, 100 ml. of water and shake vigorously for 5 minutes; then measure in 100 ml. of 1%  $\text{Na}_2\text{C}_2\text{O}_4$ , stopper, and shake 5 minutes; let stand 10 minutes, shake again for 2 minutes, and then proceed as directed in the method." I tried one determination on each sample by this procedure and obtained the following results:

Malted milk . . . . . % Casein ( $N \times 6.38$ ) 5.62

Chocolate malted milk . . . . . % Casein ( $N \times 6.38$ ) 2.18

The collaborative results and the results of the Associate Referee agree quite closely among themselves and with the calculated values for casein on both the plain malted milk and chocolate malted milk.

Although only a limited number of samples have been analyzed by the proposed method, the Associate Referee believes that the method is applicable to all malted milks, either true or synthetic, and therefore recommends\* that the proposed method be adopted as tentative.

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 56 (1941).

## REPORT ON DRIED MILK

By FRED HILLIG (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Pursuant to the recommendation made at last year's meeting that the study of methods for the determination of lactic acid in dried milk be continued, the Associate Referee made a comparison of the Troy-Sharp aldehyde method<sup>1</sup> and the colorimetric method proposed by the Associate Referee, *This Journal*, 20, 130 (1937).

TABLE 1.—*Lactic acid in dried skim milk*

SAMPLE NO.	CONDITION OF MILK	ALDEHYDE METHOD	COLORIMETRIC METHOD
		<i>per cent</i>	<i>per cent</i>
1	Not Neutralized	0.053 0.049	0.042 0.042
2	Not Neutralized	0.081 0.082	0.079 0.079
3	Not Neutralized	0.208 0.202	0.198 0.198
4	Not Neutralized	0.351 0.351	0.342 0.352
5	Not Neutralized	0.841 0.851	0.882 0.882
6	Neutralized	0.436 0.446 0.432 0.425	0.494 0.498 0.495 0.494
7	Neutralized	3.380 3.351	3.576 3.596
8	Neutralized	1.937 1.931	2.260 2.238

Briefly, the principal steps in the aldehyde method are the following: precipitation of the interfering materials with cupric hydroxide at 45° C., oxidation of the filtrate with sulfuric acid-manganous sulfate and potassium permanganate, removal of the acetaldehyde by distillation into a sodium sulfite solution, and titration of the bound sulfite with iodine.

The principal steps in the colorimetric method are precipitation of the protein with sulfuric and phosphotungstic acids, extraction of the filtrate in a continuous extractor with ether, precipitation of the citric acid with barium hydroxide in 80 per cent alcohol, evaporation of the alcohol,

<sup>1</sup> Cornell Univ. Agr. Exp. Sta. Memoir 179, June 1935.

clarification with charcoal, and reading of the color produced with ferric chloride in a photometer.

Skim milk powders of known origin were chosen for the work. The results are given in the tables.

It will be seen that when the two methods were applied to unneutralized dry skim milk they gave closely agreeing results. However, in the case of

TABLE 2.—*Lactic acid in dry milk*

SAMPLE NO.	CONDITION OF MILK	ALDEHYDE METHOD	COLORIMETRIC METHOD
1	Not Neutralized	<i>per cent</i>	<i>per cent</i>
		0.539	0.548
		0.549	0.538
2	Neutralized	0.460	
		0.449	0.532
		0.448	0.532
		0.458	

neutralized, dry, skim milks more lactic acid was returned when the colorimetric method was used than when the aldehyde method was employed.

In Table 2 data are presented on the determination of lactic acid in two dry skim milks prepared from the same batch of liquid milk. The milk was allowed to sour, one portion was dried, and the other portion was neutralized and dried.

Both methods returned the same quantity of lactic acid in the un-

TABLE 3.—*Collaborative results on lactic acid in dry milk*

SAMPLE NO.	CONDITION OF MILK	ALDEHYDE METHOD	COLORIMETRIC METHOD
1	Not Neutralized	<i>per cent</i>	<i>per cent</i>
		0.126	0.104
2	Neutralized	0.116	0.108
		0.295	0.341
		0.295	0.341

neutralized sample (Table 2). The lactic acid determined by the colorimetric method in the neutralized sample checked that obtained by both methods in the unneutralized sample. However, the aldehyde method applied to the neutralized sample failed to return all the lactic acid.

In the Associate Referee's report on neutralizers in dairy products, *This Journal*, 22, 496 (1939), collaborative results are given on the determination of lactic acid in dried skim milk by both methods. In this report it was pointed out that the aldehyde method yielded fairly satisfactory

results and that the results obtained by the colorimetric method were not satisfactory. The results obtained by the Associate Referee, using the colorimetric method, and those obtained by a commercial chemist thoroughly familiar with the aldehyde method, are given for comparison (Table 3).

Here again satisfactory results were obtained by the use of both methods on the unneutralized milk. However, the colorimetric method, as shown previously, returned more lactic acid when applied to the neutralized milk than did the aldehyde method.

In view of the discordant results obtained on the neutralized dry milk samples, it is recommended that further work be done.

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No report on lactose in milk was given by the associate referee.

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### REPORT ON MOLD IN BUTTER

By J. D. WILDMAN (Washington, D. C.), *Associate Referee*, and E. W. COULTER (Chicago, Ill., U. S. Food and Drug Administration)

This report covers collaborative work done during four years on the method for determining mold mycelia in butter that was adopted as tentative by the Association in 1938. The following analysts from the Food and Drug Administration did collaborative work:

E. W. Coulter, Chicago Station  
M. S. Goodman, Chicago Station  
H. G. Underwood, Chicago Station  
J. Carol, Chicago Station  
H. I. Macomber, Baltimore Station  
F. Allen Hodges, Microanalytical Division  
J. D. Wildman, Microanalytical Division

The results obtained on 151 investigational samples and 83 official samples are shown in the tables. To facilitate comparison both the actual counts and the differences between the counts are given. When each of two analysts make one count on a sample one difference is possible, but if more than two analysts count a sample the number of possible differences is increased in proportion to the number of analysts and the number of counts made by each. The differences given are positive gross differences, and no distinction is made as to whether the second analyst is higher or lower than the first analyst. The average difference between two analysts calculated algebraically; that is, with respect to whether the second analyst is higher or lower than the first, is an entirely different figure, which may be expected to approach zero as the number of samples on which the average is based is increased, provided the two analysts are counting alike. The average gross difference, on the other hand, reflects more particularly the extent of variation in results.

TABLE 1.—*Comparison of counts by two or more analysts  
(Mold counts from 0–5% and from 95.1–100%.)  
(50 fields)*

SUB	MOLD COUNTS		DIFFERENCE	SUB	MOLD COUNTS		DIFFERENCE
	ANALYST				ANALYST		
	A	B			A	B	
2	2	0	2	48	0	0	0
5	2	2	0	49	0	0	0
9	6	0	6	50	4	0	4
10	0	0	0	51	2	2	0
12	0	0	0	52	0	0	0
15	4	0	4	56	0	0	0
17	0	0	0	60	2	2	0
20	0	0	0	75	0	0	0
21	4	0	4	76	0	0	0
23	0	0	0	82	2	0	2
24	2	2	0	90	2	0	2
26	0	0	0	93	0	0	0
33	0	0	0	94	2	0	2
34	0	0	0	95	0	0	0
35	0	0	0	96	2	0	2
36	0	0	0	97	0	0	0
37	0	0	0	98	0	0	0
38	0	0	0	99	0	0	0
39	0	0	0	101	0	0	0
40	0	0	0	102	0	0	0
41	0	0	0	105	6	4	2
42	0	0	0	106	0	0	0
43	0	0	0	107	2	6	4
44	0	0	0				
45	0	0	0	22	100	100	0
46	0	0	0	69	94	100	6
47	0	0	0	110	98	100	2

Total number of samples—53.

Average difference between two analysts—0.79%.

(100 fields)

SUB	MOLD COUNTS					DIFFERENCES			
	ANALYST AND COUNT NO.								
	B1	B2	C1	C2	D1		D2	E	F
119	0—0		0—0		0—0		0		*
120	0—0		0—0		0—0		0		*
126	1		0—0						1, 1
135			100				100		0
145							100	98	2
150							100	93	7

Total number of samples—6

Average difference between analysts—0.27%

\* For subs 119 and 120, 36 comparisons are possible, all showing 0 difference.

TABLE 2.—*Comparison of counts by two or more analysts*  
(Mold counts between 5.1 and 25% and 75.1 and 95%.)

(50 FIELDS)				(100 FIELDS)									
SUB	MOLD COUNTS		DIFFER- ENCE	SUB	MOLD COUNTS								DIFFER- ENCE
	ANALYST				ANALYST AND COUNT NO.								
	A	B			B1	B2	C1	C2	D1	D2	E	F	
8	8	18	10	117	13—11		11—14		12—11		13		*
11	34	12	22	122	21—24		23—18				27		*
13	18	26	8	123	9—8		6—6				12		*
14	22	10	12	124	8—11		9—10				19		*
16	14	6	8	132			18				20		2
19	8	10	2	127			79				80		1
58	14	12	2	128			86				85		1
62	18	10	8	130			84				80		4
64	18	10	8	129			86				88		2
65	6	6	0	131			82				81		1
74	18	12	6	133			89				86		3
77	16	16	0	32			89				88		1
84	30	18	12	136			90					90	0
85	12	4	8	137			87				90		3
91	18	20	2	138							90	90	0
103	4	8	4	139							90	88	2
116	12	8	4	140			85				87		2
3	80	76	4	141							85	89	4
4	90	84	6	142							92	90	2
27	76	78	2	144							91	86	5
28	92	94	2	146							98	91	7
29	90	72	18	147							80	93	13
53	76	78	2	148							79	82	3
54	76	80	4	149							85	78	7
55	74	82	8										
66	92	92	0										
71	94	94	0										
92	18	34	16										
113	76	82	6										
115	78	78	0										

Total number of samples—30

Average difference between  
two analysts—6.13

Total number of samples—24

Average difference between  
analysts—3.19

\* For differences on Subs 117, 122, 123, and 124, see Table 2A.

In the largest single group of samples, Goodman and Coulter counted 50 fields on each sample. In the remaining samples 100 fields were counted, and in a number of these cases more than two analysts participated. In each table the results are presented on the basis of either 50 or 100 fields.

In addition to a separation of the counts on the basis of number of fields counted, the results are further segregated on the basis of the magnitude of the count as follows:

Table 1	{ All counts between 0% and 5%.
	{ All counts between 95.1% and 100%.
Table 2	{ All counts between 5.1% and 25%.
	{ All counts between 75.1% and 95%.
Table 3	(All counts between 25.1% and 75%.

If the counts did not fall within the limits given, the average count was considered. If more than two analysts were involved, the differences are shown in Tables 2A and 3A.

TABLE 2A.—*Possible differences among analysts when more than two analysts reported results*

(Mold counts between 5.1 and 25% and 75.1 and 95%.)

POSSIBLE COMBINATIONS OF ANALYSTS	SUB			
	117	122	123	124
C1 and B1	2	2	3	1
C1 and B2	0	1	2	2
C2 and B1	1	3	3	2
C2 and B2	3	6	2	1
C1 and D1	1			
C1 and D2	0			
C2 and D1	2			
C2 and D2	3			
C1 and E1	2	4	6	10
C2 and E1	1	9	6	9
B1 and D1	1			
B1 and D2	2			
B2 and D1	1			
B2 and D2	0	6		
B1 and E1	0	6	3	11
B2 and E1	2	3	4	9
D1 and E1	1			
D2 and E1	2			

It will be noted from Table 4 that the average difference between two analysts increases as the counts approach 50 per cent, regardless of whether 50 fields or 100 fields were counted, but that for each class of counts the average difference between two analysts decreases as the number of fields is increased. Both of these trends are to be expected from a consideration of the sampling relationship given by the following equation:



$$S.D. = \sqrt{\frac{PQ}{n}},$$

in which S.D. = standard deviation;

$P$  = percentage or average count;

$Q = 100 - P$ ; and

$n$  = number of units involved (fields).

A previous study, *This Journal*, 14, 4, 563-570 (1931), shows that the above equation is fundamental to sampling problems similar to those involved in the Howard method. While some differences between analysts appear to be unduly large it is likely that these can be accounted for by the basic sampling relationship, although it is possible that differences in individual technic may account for some of them. The accuracy of the

TABLE 3.—Comparison of counts by two or more analysts  
(Mold counts between 25.1-75 %.)

SUB	MOLD COUNTS 50 FIELDS		DIFFERENCES
	ANALYST		
	A	B	
1	58	52	6
6	52	60	8
7	38	40	2
18	66	66	0
25	58	58	0
30	66	68	2
31	30	22	8
57	38	46	8
59	34	22	12
61	42	50	8
63	20	36	16
67	36	26	10
68	78	66	12
70	64	58	6
72	52	40	12
73	40	42	2
78	36	38	2
79	36	24	12
80	34	44	10
81	46	32	14
83	54	52	2
86	52	54	2
87	30	30	0
88	28	34	6
89	30	30	0
100	52	60	8
104	56	54	2
108	42	26	16
109	26	36	10
111	46	34	12
112	72	74	2
114	38	40	2

Total No. samples—32

Average difference between analysts—6.62

TABLE 3.—*Continued*

SUB	MOLD COUNTS (100 FIELDS)					DIFFERENCE
	ANALYST AND COUNT NO.					
	B1 B2	C1 C2	D1 D2	E	F	
118	61—58	60—56	64—62	45		*
121	49—53	47—48	47—49	41		*
125	37—38	32—35		40		*
134		72		67		5
143				72	71	1
151				64	67	3

Total No. samples—6

Average difference between analysts—5.43

\* For differences on Subs 118, 121, and 125, see Table 3A.

TABLE 3A.—*Possible differences among analysts when more than two report results*  
(Mold counts between 25.1 and 75%.)

POSSIBLE COMBINATIONS OF ANALYSTS	SUB		
	118	121	125
C1 and B1	1	2	5
C1 and B2	2	6	6
C2 and B1	5	1	2
C2 and B2	2	5	3
C1 and D1	4	0	
C1 and D2	2	2	
C2 and D1	8	1	
C2 and D2	6	1	
C1 and E1	15	6	8
C2 and E1	11	7	5
B1 and D1	3	2	
B1 and D2	1	0	
B2 and D1	6	6	
B2 and D2	4	4	
B1 and E1	16	8	3
B2 and E1	13	12	2
D1 and E1	19	6	
D2 and E1	17	8	

For an easy comparison of the average differences between two analysts, Table 4 was prepared.

TABLE 4.—*Comparison of average differences between two analysts*

NO. OF FIELDS COUNTED	MAGNITUDE OF COUNTS (%)		
	0-5	5.1-25	25.1-75
	95.1-100	75.1-95	
50	0.79	6.13	6.62
100	0.27	3.19	5.43

method depends primarily upon the number of fields counted provided the analyst is thoroughly qualified for the work. Good results can not be obtained by an analyst unfamiliar with the requirements of the method.

In Table 5 are given the results obtained by two analysts on official samples involving 83 churns of butter with each analyst counting a sample from a different tub from the same churn and using 100 fields. In general the counts themselves were high. It will be noted that generally there is good agreement among the analysts.

It is recommended that the mold mycelia method be adopted as official, first action.

TABLE 5.—*Comparison of differences between two analysts on official samples (100 fields counted.)*

DEVIATION IN RESULTS ON DIFFERENT TUBS BY DIFFERENT ANALYSTS	NO. OF CHURNS	PER CENT CHURNS
0	20	24
1	11	13
2	11	13
3	14	17
4	6	7
5	3	4
6	4	5
7	7	9
8	4	5
9	0	0
10	2	2
Over 10 (12)	1	1
	83	100

No report on decomposition in dairy products was given by the referee.

## REPORT ON NEUTRALIZERS IN DAIRY PRODUCTS

By FRED HILLIG (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Pursuant to the recommendation made at last year's meeting that ash alkalinity studies on dairy products be continued, the Associate Referee made further investigation of the Tillmans-Bohrmann<sup>1</sup> method for the determination of ash alkalinity. In this method the effect of phosphates is eliminated by the addition of calcium chloride to the acidified ash prior to back titration with standard alkali. The method was found to be easy

<sup>1</sup> *Z. Nahr. Genussm.*, 41, 1-17 (1921).

of manipulation, and satisfactory results were obtained by its use. The method follows.

#### ALKALINITY OF ASH OF DRY SKIM MILK

Ash 2 grams of dry skim milk for 1 hour at 550° C. Add a few ml. of water to the ash, break up with a flattened stirring rod, evaporate to dryness on the steam bath, and again ash for 1 hour. Again add a few ml. of water to the ash, break up, and transfer

TABLE 1.—*Alkalinity of ash and water-soluble ash of dry skim milk*

SAMPLE	ALKALINITY OF WATER-SOLUBLE ASH	ALKALINITY OF ASH
	ml. 0.1 N/100 g.	
1	24	93
	25	98
2	25	109
	25	105
3	20	115
	20	105
4	25	98
	25	100
5	20	85
	18	85
6	25	90
	25	95
7	15	80
	13	80
8	26	96
	29	96
9	28	115
	25	115
10	25	100
	25	100
11	25	106
	25	101
12	25	105
	25	100
13	21	104
	21	104
14	20	95
	20	90
Average	23	99

to a beaker with 50–75 ml. of water. Add 50 ml. of 0.1 *N* HCl, heat to boiling, and boil gently for 5 minutes. Cool, add 30 ml. of a 40% solution of CaCl<sub>2</sub> (neutralized with 0.1 *N* HCl and filtered) and about 10 drops of phenolphthalein indicator, and titrate the excess acid with 0.1 *N* NaOH. Acid used (ml.)  $\times 50$  = alkalinity of ash of dry skim milk.

In the Associate Referee's report to the Association in 1939, *This Journal*, 22, 496 (1939), results obtained by collaborators on the determination of the alkalinity of water-soluble ash in several samples of dry skim milk were given. While individual analysts were able to obtain good check determinations, comparison of the results of the various analysts showed that they were far from satisfactory. The temperature at which the ash was extracted and the filtering and washing technic used might possibly have been the cause of the poor results obtained. It was thought that if these steps in the procedure could be standardized, satisfactory determinations of the alkalinity of the water-soluble ash would be possible. With this in mind, the Associate Referee devised the following method.

#### ALKALINITY OF WATER-SOLUBLE ASH OF DRY SKIM MILK

Ash 2 grams of dry skim milk as directed in previous method, and transfer the ash to a beaker with 50 ml. of water. Heat to boiling and boil gently for 10 minutes, adding water to keep the volume approximately 50 ml. Transfer the contents of the beaker to a 100–110 ml. volumetric flask, cool, make to 110 ml., and filter. Pipet 100 ml. of the clear filtrate into a beaker, add 25 ml. of 0.1 *N* HCl, heat to boiling, and boil gently for 5 minutes. Cool, and titrate the excess acid with 0.1 *N* NaOH, using phenolphthalein indicator. (Acid used (ml.)  $\times 1.1$ )  $\times 50$  = alkalinity of water-soluble ash of dry skim milk.

The alkalinity of the ash and the water-soluble ash were determined on a number of unneutralized dry skim milks of good quality. The results are given in Table 1.

TABLE 2.—*Alkalinities of ash of neutralized dry skim milks*

SAMPLE	ALKALINITY OF WATER-SOLUBLE ASH	ALKALINITY OF ASH
	ml. 0.1 <i>N</i> /100 g.	
1	44	229
	41	234
2	53	241
	50	235
3	69	306
	68	309
4	206	497
	200	504
5	203	499
	210	490

The results show that the ash in the dry milks examined is quite uniform as regards alkalinities.

Alkalinity determinations were also made on several milks known to have been neutralized. The results are given in Table 3.

The alkalinities of the ash of the neutralized dry milks examined are several times greater than those of the unneutralized dry milks.

The methods were also applied to a reconstituted dry skim milk of good quality to which sodium bicarbonate in varying quantities had been added. When the alkalinities of the ash were determined, due allowance being made for the alkalinity of the milk itself, good recoveries of the added bicarbonate were obtained. However, contrary to all expectations all the added bicarbonate was *not* returned as alkalinity of the water-soluble ash, the maximum recovery being about 50 per cent. This matter is now being investigated.

During the coming year the two alkalinity methods will be submitted to collaborative study. It is recommended that the work be continued.

## REPORT ON TESTS FOR PASTEURIZATION OF DAIRY PRODUCTS

### PHOSPHATASE TEST IN EXAMINATION OF MILK AND CREAM

#### III. RAPID (LABORATORY) TEST

#### IV. FURTHER STUDY OF TENTATIVE METHOD

#### V. FURTHER STUDY OF TECHNIC FOR USE IN THE FIELD

By F. W. GILCREAS (From the Division of Laboratories and  
Research, New York State Department of Health,  
Albany, N. Y.), *Associate Referee*

#### III. RAPID (LABORATORY) TEST

A rapid phosphatase test to be used in the laboratory for the detection of pasteurization of milk has been developed in the chemical laboratories of the New York City Department of Health (1, 2). The sample is incubated for one hour at 37° C. following the addition of a buffered solution of disodium phenyl phosphate. The phenol liberated is estimated by the use of an alcoholic solution of 2,6 dibromoquinonechloroimide. The procedure has been offered as equivalent in precision and sensitivity to the tentative standard method of this Association (3a). Since a test of this character would be of great value in the control of pasteurization, the Associate Referee decided to study the test in collaborative examination, as he had done with other forms of the phosphatase test. The latest technic was used, that published in the 7th edition of "Standard Methods for the Examination of Dairy Products," of the American Public Health Association (3b).

Beginning March 5, 1940, the following ten laboratories examined weekly eight series of twelve undesignated samples each.

(1) Bureau of Milk Sanitation, New York State Department of Health, Albany (Mobile Laboratories, Nos. 1 and 2), W. D. Tiedeman, Chief.

(2) Bureau of Laboratories, Connecticut State Department of Health, Hartford, F. L. Mickle, Director.

(3) New York State Agricultural Experiment Station, Division of Dairying, Geneva, R. F. Holland, Director.

(4) The von Wedel Laboratories, New Rochelle, N. Y., H. von Wedel, Director.

(5) Connecticut State College, Storrs, E. O. Anderson, Associate Professor Dairy Industry.

(6) Tompkins County Laboratory, Ithaca, N. Y., H. W. Ferris, Director.

(7) New Jersey Agricultural Experiment Station, New Brunswick, O. F. Garrett, Assistant Professor of Dairy Manufactures.

(8) Research Laboratories, Sealtest, Inc., Baltimore, Md., J. J. Johnson.

(9) New York State Department of Agriculture and Markets, State Food Laboratory, Albany, C. E. Safford, Bacteriologist.

(10) Chemical Laboratory, New York City Department of Health, Jerome Trichter, Acting Director Bureau of Food and Drugs.

Each laboratory prepared its own reagents and color standards. The acid series of color standards was used since it covers the range of phosphatase values for underpasteurization frequently encountered. At the close of the study the standards were sent to the Associate Referee to be compared with those prepared in his laboratory.

The samples represented milk commercially pasteurized at 143° F. for varying lengths of time, pasteurized milk and cream to which known quantities of raw milk had been added, and milk pasteurized in the laboratory at 142° F. for varying periods. Shipment was made by parcel post, special delivery, without refrigeration, which seemed satisfactory at the prevailing northern temperatures of March and April. The samples were in transit less than 24 hours, and all the laboratories reported that they had been received in a condition satisfactory for examination.

The results of the study of this modification of the phosphatase test are given in Table 1 and Figures 1 and 2. The frequencies are given per half rather than full unit in order to take into account the unequal intervals between standards. Samples accorded the same treatment and having the same first digit in the sample number are replicates from the same preparation. In estimating frequencies and medians, and in all graphic representations only the first result obtained was used. The unshaded portions indicate the median values. In 9 of 95 observations, milk heated to 142° F. for 30 minutes was incorrectly recorded as completely pasteurized. Milk that had been heated for 15 and 20 minutes at 143° F. was selected correctly in 93 and 79 observations, respectively, out of a total of 96. Pasteurized milk was detected in all but two of 96 observations. These findings indicate that the test compares favorably in accuracy with the tentative standard procedure.

TABLE 1.—*Results of examination of prepared samples by rapid (laboratory) test*  
(Results expressed in phosphatase units)

SAMPLE NUMBER	COOPERATING LABORATORIES											
	1	2	3	4	5	6	7	8	9	10	11	12

*Milk held at 142° F. for 25 minutes*

110	5.0	5.0	6.5	5.0	4.25	4.5	7.5	5.0	6.0	5.0	5.0	1.5
206	7.5	5.0	7.2	10.0	4.25	4.0	10.0	7.5	8.5	2.0	5.0	9.0
303	5.0	7.5	6.8	6.25	4.25	6.0	10.0	5.0	7.0	5.0	6.25	10.0
402	8.0	5.0	8.0	6.25	6.25	5.5	10.0	7.5	8.5	4.0	5.0	10.0
508	6.0	7.5	5.2	4.25	2.75	4.5	7.5	5.0	2.5	6.0	10.0	6.0
602	4.0	6.0	4.3	4.25	2.75	9.0	6.0	5.0	6.0	4.0	10.0	6.0
707	8.0	7.5	7.0	5.0	4.25	8.0	10.0	7.5	9.0	6.0	6.25	3.5
801	6.0	6.0	5.1	6.25	2.75	8.5	10.0	10.0	7.5	10.0	5.0	7.5

*Milk held at 142° F. for 30 minutes*

106	3.5	3.5	2.5	2.75	1.5	2.5	4.0	3.5	4.0	4.0	3.5	1.0
202	4.0	2.5	4.0	5.0	2.75	3.0	3.5	5.0	5.0	2.0	3.5	5.0
306	4.0	3.5	3.8	3.5	2.75	4.5	5.0	3.5	4.5	4.0	3.5	4.5
408	4.0	3.5	4.8	3.5	4.25	4.0	6.0	5.0	4.0	2.0	2.75	4.0
503	4.0	4.0	4.3	2.0	1.5	3.8	5.0	3.5	4.5	4.0	7.5	
608	3.5	3.5	3.5	2.0	2.75	5.5	5.0	3.5	3.5	3.0	8.75	5.0
702	4.0	3.5	3.5	4.25	1.5	5.0	5.0	5.0	5.0	2.0	4.25	2.0
806	4.0	3.6	3.8	3.5	1.5	4.5	5.0	5.0	4.5	7.0	5.0	4.0

*Milk held at 143° F. for 15 minutes*

105	7.5	12.0	7.0	10.0	4.25	5.0	10.0	10.0	8.0	6.0	5.0	1.5
201	6.5	4.0	7.0	10.0	4.25	4.5	10.0	7.5	8.0	2.0	5.0	7.5
203	6.5	4.0	7.0	8.75	4.25	4.5	5.0	7.5	7.5	5.0	7.5	9.0
312	8.0	7.5	8.0	6.25	6.25	6.5	10.0	5.0	8.5	7.0	6.25	10.0
407	8.0	9.0	8.0	7.5	6.25	6.0	10.0	7.5	8.5	4.0	7.5	10.0
510	8.0	10.0	7.5	5.0	4.25	6.0	10.0	10.0	8.5	8.0	10.0	6.0
604	8.0	10.0	8.5	6.25	8.75	10.0	10.0	15.0	9.0	6.0	12.5	9.0
709	8.0	10.0	7.2	6.25	4.25	8.0	10.0	10.0	9.0	6.0	6.25	8.0
712	8.0	10.0	6.2	4.25	2.75	8.0	10.0	10.0	8.5	6.0	6.25	8.0
803	6.0	6.0	5.2	4.25	1.50	8.0	10.0	10.0	7.0	10.0	6.25	7.0

*Milk held at 143° F. for 20 minutes*

101	3.5	3.5	1.5	8.75	1.0	3.5	5.0	3.5	4.0	4.0	3.5	1.0
112	2.5	3.5	1.8	3.5	2.75	2.5	4.0	3.5	4.0	4.0	3.5	1.0
208	3.5	2.0	2.6	4.25	2.75	1.0	4.0	3.5	4.5	2.0	2.0	4.0
310	3.5	3.5	4.0	2.75	1.5	4.0	5.0	3.5	5.0	2.0	2.75	4.0
404	4.0	3.5	3.5	2.75	1.5	3.5	5.0	3.5	4.5	2.0	3.5	0.0
505	4.0	4.0	3.8	2.0	1.5	3.9	5.0	5.0	4.5	4.0	7.5	4.0
512	4.0	4.0	4.0	2.75	1.5	4.0	6.0	3.5	4.0	3.0	7.5	3.5
610	4.0	3.5	4.0	4.25	2.75	6.0	5.0	3.5	4.5	3.0	10.0	6.0
704	3.5	3.0	4.3	4.25	1.5	4.5	3.5	3.5	4.5	2.0	5.0	2.0
808	3.5	2.0	1.2	3.5	1.5	4.0	5.0	2.0	4.0	5.0	3.5	3.5



TABLE 1.—Continued

SAMPLE NUMBER	COOPERATING LABORATORIES											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Milk held at 143° F. for 25 minutes</i>												
108	1.5	2.0	1.0	2.0	1.5	1.5	2.0	1.0	2.5	2.0	2.75	0.0
204	1.5	1.0	1.3	2.0	2.75	1.0	3.5	2.0	3.0	1.0	2.0	2.0
301	1.5	1.5	1.5	2.5	1.5	1.7	4.0	2.0	3.0	1.0	2.0	2.0
410	1.5	1.0	1.8	1.5	1.5	1.3	3.5	1.0	2.0	1.0	1.5	2.0
511	1.5	2.0	1.6	1.5	1.0	1.5	3.5	2.0	2.0	2.0	3.5	1.5
605	1.5	2.0	1.5	2.0	1.5	3.0	4.0	2.0	3.0	2.0	5.0	4.0
710	1.5	2.0	1.0	1.5	1.5	2.0	2.0	1.0	4.0	1.0	2.0	1.0
804	1.5	1.0	1.2	2.0	1.0	2.5	2.0	1.0	3.5	4.0	2.0	2.0
<i>Milk held at 143° F. for 30 minutes</i>												
104	1.0	1.0	1.0	1.5	1.0	1.0	0.5	0.0	1.0	1.0	1.5	1.0
211	1.5	1.0	1.0	1.5	1.0	1.5	1.0	1.0	1.5	1.0	1.5	0.0
309	1.0	1.0	1.5	1.5	1.0	1.5	2.0	0.0	3.5	2.0	1.5	1.0
411	1.5	1.0	1.3	1.0	1.0	1.0	2.0	1.0	1.5	1.0	1.5	0.0
506	1.5	1.0	1.4	1.0	1.0	1.3	0.0	0.0	1.5	1.0	2.0	1.0
611	1.5	1.5	1.0	1.0	1.5	1.5	3.5	1.0	1.5	1.0	1.5	1.5
705	1.5	1.0	1.5	1.5	1.5	1.0	0.0	1.0	1.5	1.0	2.0	0.0
809	1.0	1.0	1.0	1.0	1.0	1.5	1.0	0.0	2.0	1.0	2.0	2.0
<i>Light cream prepared from heavy cream held at 145° F. for 15 minutes plus milk held at 143° F. for 15 minutes</i>												
305*	1.5	1.0	1.5	1.5	1.0	3.0	3.5	0.0	2.0	1.0	1.5	1.0
311*	1.5	1.0	1.5	1.0	1.0	1.5	2.0	1.0	3.5	1.0	1.5	1.0
409	4.0	4.0	4.0	2.75	4.25	4.0	7.5	3.5	4.5	2.0	3.5	5.0
507	4.0	4.0	4.2	3.5	1.5	4.0	6.0	3.5	4.0	3.0	8.75	4.0
601	4.0	6.0	4.5	5.0	4.25	9.0	6.0	5.0	6.0	3.0	10.0	6.0
706	4.0	4.0	4.8	4.25	1.5	5.5	5.0	5.0	6.0	4.0	4.25	3.5
811	4.0	3.5	3.0	4.25	1.5	4.5	5.0	3.5	4.0	6.0	3.5	4.0
<i>Light cream prepared from heavy cream held at 145° F. for 30 minutes plus milk held at 143° F. for 30 minutes</i>												
103	1.0	1.0	1.0	1.5	1.0	1.0	0.5	0.0	1.0	1.0	1.5	0.0
210	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.5	1.0	2.0	0.0
307	1.0	1.0	1.5	1.0	1.0	2.0	0.0	0.0	1.5	1.0	1.0	1.0
406	1.0	1.0	1.0	1.0	1.0	1.0	2.0	0.0	1.5	1.0	1.0	0.0
502	1.0	1.0	1.3	1.0	1.0	1.3	0.0	0.0	1.5	1.0	1.5	1.5
607	1.0	1.0	1.0	1.0	1.0	1.5	3.5	0.0	1.5	1.0	1.5	2.0
701	1.5	1.0	1.0	1.5	1.0	1.2	0.0	0.0	1.5	1.0	1.5	0.0
805	1.5	1.0	1.0	1.0	1.0	1.5	0.0	0.0	1.5	1.0	1.0	1.0
<i>Milk held at 143° F. for 30 minutes plus 0.5% raw milk</i>												
102	3.5	3.0	2.0	2.75	2.0	3.5	5.0	3.5	4.0	3.0	3.5	1.5
107	3.5	4.0	2.5	3.5	2.75	2.5	4.0	3.5	4.5	4.0	4.25	1.0
209	4.0	3.0	3.6	5.0	2.75	2.5	6.0	5.0	6.0	1.0	3.5	5.0
212	4.0	2.0	4.0	4.25	2.75	3.5	6.0	5.0	5.5	3.0	4.25	5.0
302	4.0	4.0	4.0	4.25	2.75	5.0	7.5	3.5	4.5	4.0	3.5	5.0
405	3.5	3.5	4.0	4.25	2.75	2.5	5.0	3.5	4.0	2.0	2.75	1.0
509	5.0	5.0	5.0	4.25	1.5	4.3	7.5	5.0	5.0	5.0	10.0	4.0
603	3.5	5.0	2.0	1.5	1.5	5.5	5.0	5.0	4.5	3.0	7.5	5.0
612	4.5	5.0	4.0	2.0	4.25	5.5	5.0	5.0	4.5	4.0	10.0	5.0
708	5.0	5.0	4.3	4.25	1.5	6.0	7.5	5.0	6.0	4.0	6.25	3.5
802	4.0	3.5	3.7	3.5	1.5	4.5	5.0	3.5	4.5	6.0	3.5	4.0

TABLE 1.—Continued

SAMPLE NUMBER	COOPERATING LABORATORIES											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Milk held at 143° F. for 30 minutes plus 0.15% raw milk</i>												
109	1.5	3.0	1.0	2.0	1.5	2.5	3.5	2.0	3.5	3.0	2.75	1.0
205	3.5	2.0	1.5	3.5	2.75	1.0	5.0	3.5	4.0	1.0	2.0	4.0
308	3.5	2.5	3.8	4.25	1.5	4.0	4.0	2.0	4.0	2.0	2.0	3.5
401	1.5	2.0	3.0	2.0	1.5	1.5	4.0	2.0	3.0	1.0	2.75	2.0
504	4.0	2.0	4.2	1.5	1.5	3.8	5.0	3.5	4.5	1.0	6.25	2.0
609	2.0	3.0	3.4	2.0	1.5	4.0	4.0	2.0	3.0	4.0	7.5	4.0
703	3.5	3.0	1.8	4.25	1.5	4.5	3.5	3.5	4.5	2.0	3.5	2.0
807	2.0	1.5	1.2	2.0	1.5	4.0	3.5	2.0	3.5	4.0	3.5	3.5
<i>Light cream prepared from heavy cream held at 145° F. for 30 minutes plus milk held at 143° F. for 30 minutes plus 0.3% raw milk</i>												
111	2.5	4.0	2.0	4.25	2.75	2.5	0.0	2.0	4.0	4.0	4.25	1.0
207	4.0	2.0	4.1	4.25	2.75	2.5	5.0	3.5	5.0	1.0	3.5	4.0
304	4.0	3.5	4.1	5.0	2.75	4.5	6.0	3.5	4.5	2.0	3.5	5.0
403	3.5	2.0	3.0	2.75	1.5	3.0	5.0	2.0	4.5	1.0	2.75	4.0
412	3.5	2.0	3.5	2.75	4.25	3.5	5.0	3.5	3.5	2.0	3.5	4.0
501	5.0	5.0	5.5	3.5	1.5	4.5	7.5	5.0	6.0	4.0	10.0	4.0
606	3.5	5.0	2.2	2.0	1.5	5.5	5.0	3.5	4.0	2.0	8.75	5.0
711	5.0	5.0	4.0	4.25	1.5	6.0	6.0	5.0	6.5	4.0	5.0	2.0
810	4.0	3.5	3.5	2.75	1.5	4.5	5.0	3.5	4.5	5.0	3.5	4.0
812	4.0	3.0	3.0	2.0	1.5	4.5	5.0	3.5	4.5	5.0	3.5	4.0

\* Prepared with milk held at 143° F for 30 minutes.

TABLE 2.—Comparison of acid series color standards for rapid (laboratory) test

LABORATORY	COLOR VALUES					
	1 UNIT	2 UNITS	3.5 UNITS	5 UNITS	7.5 UNITS	10 UNITS
Referee	1	2	3.5	5	7.5	10
No.						
2	1	2	3.5	5	7.5	10
3		3.5	3.5	6	8	10
4	1	2	3.5	5	7.5	10
5	1	3.5	3.5	5	7.5	10
6	1	2	3.5	5	7.5	10
7	1	1.5	2	4	5	6
8	1	2	3.5	5	7.5	10
9	1	2	3.5	4.5	7.5	10
10	2	4	3.5	6	7.5	10
11	1	2	3.5	5.5	8	10
12	1	2	3.5	5	7.5	10

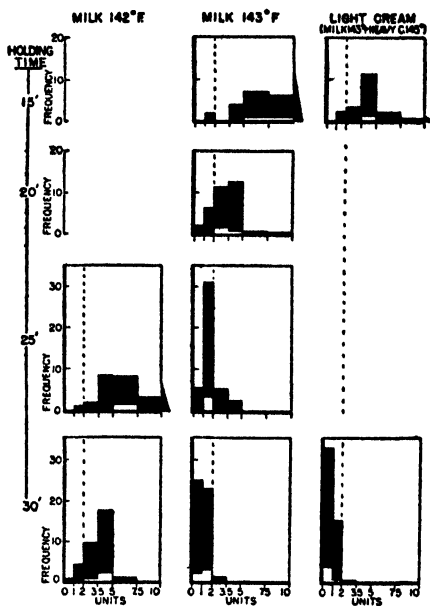


FIG. 1

FIG. 1.—RAPID (LABORATORY) PHOSPHATASE TEST.

Frequency distribution per half unit of observed readings by comparison with color standards for milk and cream heated for the given time at the given temperature.

Corresponding frequency of values >10 is equal to area of triangle. Unshaded portions indicate median values.

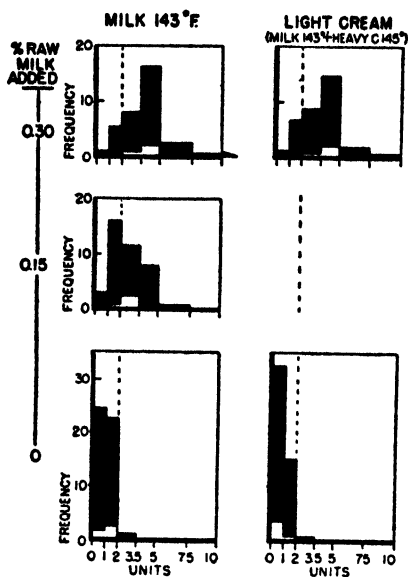


FIG. 2

FIG. 2.—RAPID (LABORATORY) PHOSPHATASE TEST.

Frequency distribution per half unit of observed readings by comparison with color standards for pasteurized milk and cream with added raw milk.

Corresponding frequency of values >10 is equal to area of triangle. Unshaded portions indicate median values.

Results of the comparison of the color standards are given in Table 2. Some of the laboratories reported difficulty with the standards prescribed by Scherer for this test. Their experience suggests that improvement in this regard might result in more precise readings.

#### RECOMMENDATION

Based on the data presented, it is recommended that the rapid (laboratory) phosphatase test developed by the New York City Department of Health be adopted as a tentative method.

#### IV. FURTHER STUDY OF THE PRESENT TENTATIVE METHOD

Since the Association's present tentative method for the phosphatase test is now used quite generally, it seemed desirable to re-examine the technic at the same time investigation was being made of the rapid lab-

oratory test. Accordingly, the same laboratories examined the samples by this method also (3a). Each laboratory prepared its own reagents, as well as its own color standards, which were checked in the Associate Referee's laboratory at the completion of the study.

Table 3 and Figures 3 and 4 show the results of the examination. Results obtained with replicate samples were ignored. The unshaded areas represent the median values for the various samples. The method failed to differentiate milk heated to 142° F. for 30 minutes in 8 of 94 observations. Milk that had been heated at 143° F. for 20 and 15 minutes was selected correctly in 81 of 95 and in 94 of 95 observations, respectively. In the examination of milk heated for 25 minutes at 143° F., 84 of 95 observations recorded it as properly pasteurized. However, a long period of preheating such as is used in the commercial plant from which the samples were taken may result in a sample indicative of treatment at

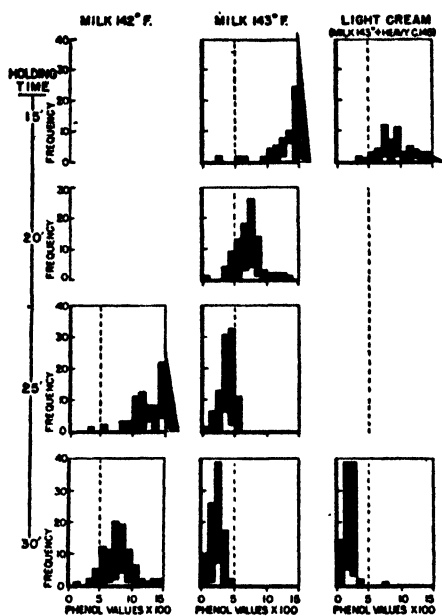


FIG. 3

FIG. 3.—PRESENT TENTATIVE METHOD.

Frequency distribution of observed phenol values (expressed as mg. per 0.5 ml. of sample) for milk and cream heated for the given time at the given temperature.

Frequency of values >0.15 proportional to area of triangle. Unshaded portions indicate median values.

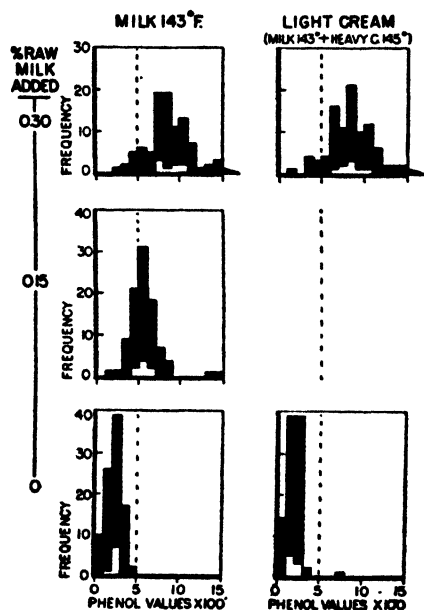


FIG. 4

FIG. 4.—PRESENT TENTATIVE METHOD.

Frequency distribution of observed phenol values (expressed as mg. per 0.5 ml. of sample) for pasteurized milk and cream with added raw milk.

Frequency of values >0.15 proportional to area of triangle. Unshaded portions indicate median values.

TABLE 3.—Results of examination of prepared samples by present tentative method  
(Results expressed in mg. of phenol per 0.5 ml. of sample.)

SAMPLE NUMBER	COOPERATING LABORATORIES											
	1	2	3	4	5	6	7	8	9	10	11	12

*Milk held at 142° F. for 25 minutes*

110	0.13	0.10	0.15	0.09		0.12	0.10	0.06	0.19	0.13	0.15	0.14
206	0.15	0.12	0.15	0.15	0.15	0.14	0.09	0.15	0.18	0.035	0.15	0.15
303	0.13	0.13	0.15	0.15	0.105	0.15	0.15	0.15	0.17	0.125	0.15	0.12
402	0.15	0.12	0.15	0.15	0.15	0.15	0.15	0.15	0.17	0.12	0.15	0.15
508	0.13	0.13	0.14	0.105	0.105	0.15	0.15	0.15	0.14	0.12	0.11	0.11
602	0.13	0.12	0.135	0.105	0.105	0.15	0.15	0.09	0.18	0.11	0.12	0.15
707	0.14	0.12	0.14	0.12	0.105	0.15	0.15	0.15	0.18	0.15	0.15	0.055
801	0.15	0.10	0.105	0.135	0.105	0.15	0.15	0.15	0.16	0.12	0.12	0.15

*Milk held at 142° F. for 30 minutes*

106	0.07	0.06	0.11	0.06		0.078	0.07	0.10	0.10	0.065	0.09	0.07
202	0.09	0.045	0.125	0.105	0.09	0.09	0.06	0.10	0.11	0.02	0.10	0.15
306	0.08	0.06	0.108	0.09	0.075	0.075	0.09	0.09	0.09	0.08	0.09	0.06
408	0.10	0.05	0.103	0.05	0.075	0.135	0.10	0.07	0.10	0.07	0.08	0.14
503	0.08	0.07	0.075	0.06	0.06	0.085	0.08	0.08	0.09	0.08	0.06	0.055
608	0.09	0.07	0.075	0.06	0.075	0.075	0.09	0.08	0.10	0.06	0.08	0.09
702	0.08	0.06	0.07		0.04	0.105	0.10	0.12	0.10	0.09	0.09	0.035
806	0.10	0.05	0.07	0.06	0.05	0.075	0.09	0.12	0.09	0.09	0.08	0.15

*Milk held at 143° F. for 15 minutes*

105	0.17	0.15	0.15	0.135		0.15	0.15	0.13	0.19	0.125	0.20	0.14
201	0.14	0.07	0.15	0.15	0.15	0.125	0.12	0.15	0.17	0.03	0.13	0.15
203	0.14	0.09	0.15	0.135	0.12	0.125	0.12	0.12	0.15	0.03	0.15	0.15
312	0.12	0.13	0.15	0.15	0.105	0.15	0.15	0.15	0.19	0.12	0.15	0.15
407	0.15	0.14	0.15	0.15	0.135	0.15	0.15	0.15	0.19	0.145	0.15	0.15
510	0.15	0.15	0.15	0.15	0.105	0.15	0.15	0.15	0.18	0.135	0.15	0.15
604	0.20	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.27	0.15	0.20	0.15
709	0.17	0.15	0.145	0.12	0.135	0.15	0.15	0.15	0.20	0.14	0.15	0.06
712	0.17	0.14	0.145	0.135	0.105	0.15	0.15	0.15	0.19	0.14	0.15	0.055
803	0.13	0.10	0.13	0.12	0.105	0.14	0.15	0.15	0.16	0.11	0.13	0.15

*Milk held at 143° F. for 20 minutes*

101	0.07	0.06	0.105	0.135		0.083	0.05	0.05	0.08	0.065	0.08	0.06
112	0.07	0.05	0.09	0.075		0.078	0.06	0.06	0.09	0.07	0.08	0.06
208	0.07	0.035	0.08	0.05	0.06	0.053	0.04	0.08	0.07	0.01	0.07	0.09
310	0.07	0.05	0.075	0.075	0.075	0.075	0.06	0.08	0.09	0.055	0.08	0.055
404	0.07	0.045	0.09	0.05	0.075	0.075	0.09	0.08	0.09	0.07	0.06	0.11
505	0.07	0.07	0.083	0.075	0.075	0.09	0.09	0.08	0.09	0.083	0.08	0.06
512	0.08	0.06	0.073	0.075	0.075	0.13	0.09	0.08	0.08	0.085	0.06	0.06
610	0.12	0.07	0.10	0.075	0.075	0.125	0.09	0.07	0.13	0.08	0.10	0.08
704	0.08	0.045	0.065	0.05	0.075	0.095	0.06	0.12	0.08	0.08	0.08	0.035
808	0.06	0.035	0.05	0.06	0.05	0.065	0.09	0.07	0.07	0.06	0.07	0.09

TABLE 3.—Continued

SAMPLE NUMBER	COOPERATING LABORATORIES											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Milk held at 143° F. for 25 minutes</i>												
108	0.04	0.03	0.045	0.04		0.052	0.02	0.04	0.05	0.05	0.045	0.03
204	0.035	0.02	0.045	0.025	0.035	0.04	0.03	0.05	0.03	0.01	0.04	0.05
301	0.045	0.025	0.05	0.06	0.035	0.04	0.05	0.04	0.05	0.035	0.04	0.04
410	0.035	0.015	0.045	0.035	0.035	0.045	0.05	0.06	0.06	0.03	0.035	0.06
511	0.045	0.025	0.038	0.05	0.025	0.055	0.05	0.05	0.05	0.05	0.035	0.03
605	0.05	0.035	0.045	0.04	0.05	0.05	0.06	0.04	0.05	0.048	0.055	0.06
710	0.045	0.04	0.035	0.035	0.05	0.045	0.04	0.06	0.02	0.04	0.04	0.03
804	0.045	0.02	0.028	0.02	0.025	0.04	0.05	0.05	0.04	0.045	0.04	0.045
<i>Milk held at 143° F. for 30 minutes</i>												
104	0.02	0.015	0.035	0.03		0.03	0.02	0.02	0.03	0.025	0.03	0.01
211	0.02	0.015	0.03	0.035	0.02	0.013	0.02	0.03	0.02	0.01	0.025	0.03
309	0.025	0.01	0.03	0.05	0.025	0.025	0.02	0.03	0.03	0.025	0.025	0.01
411	0.025	0.01	0.035	0.03	0.025	0.035	0.05	0.03	0.02	0.02	0.025	0.02
506	0.02	0.01	0.03	0.02	0.025	0.025	0.04	0.04	0.03	0.025	0.015	0.01
611	0.03	0.025	0.03	0.015	0.025	0.025	0.04	0.04	0.03	0.02	0.025	0.04
705	0.02	0.01	0.02	0.025	0.025	0.028	0.04	0.04	0.02	0.025	0.02	0.01
809	0.025	0.01	0.02	0.025		0.022	0.04	0.04	0.02	0.035	0.02	0.04
<i>Light cream prepared from heavy cream held at 145° F. for 15 minutes plus milk held at 143° F. for 15 minutes</i>												
305*	0.03	0.01	0.038	0.035	0.025	0.033	0.03	0.03	0.04	0.025	0.04	0.02
311*	0.03	0.02	0.035	0.035	0.025	0.032	0.02	0.04	0.04	0.03	0.03	0.02
409	0.10	0.06	0.103	0.075	0.075	0.135	0.08	0.08	0.10	0.07	0.08	0.15
507	0.10	0.07	0.098	0.075	0.075	0.125	0.09	0.10	0.10	0.09	0.10	0.08
601	0.13	0.12	0.135	0.105	0.105	0.15	0.15	0.09	0.17	0.10	0.13	0.15
706	0.09	0.07	0.09	0.06	0.075	0.13	0.12	0.12	0.12	0.095	0.10	0.04
811	0.10	0.05	0.07	0.06	0.04	0.065	0.09	0.12	0.08	0.08	0.09	0.14
<i>Light cream prepared from heavy cream held at 145° F. for 30 minutes plus milk held at 143° F. for 30 minutes</i>												
103	0.015	0.015	0.02	0.04		0.03	0.02	0.03	0.02	0.02	0.02	0.01
210	0.02	0.01	0.03	0.025	0.02	0.012	0.01	0.02	0.02	0.01	0.02	0.02
307	0.015	0.01	0.025	0.03	0.025	0.023	0.02	0.02	0.03	0.02	0.025	0.01
406	0.015	0.01	0.025	0.01	0.025	0.025	0.03	0.02	0.02	0.015	0.02	0.05
502	0.02	0.01	0.02	0.02	0.025	0.023	0.03	0.03	0.02	0.015	0.02	0.01
607	0.025	0.02	0.023	0.015	0.025	0.023	0.03	0.03	0.03	0.02	0.015	0.03
701	0.015	0.01	0.01	0.015	0.075	0.023	0.03	0.03	0.01	0.025	0.02	0.02
805	0.02	0.01	0.018	0.04	0.025	0.022	0.03	0.03	0.03	0.025	0.015	0.03
<i>Milk held at 143° F. for 30 minutes plus 0.3% raw milk</i>												
102	0.08	0.05	0.10	0.075		0.084	0.07	0.05	0.11	0.08	0.08	0.06
107	0.07	0.10	0.11	0.075		0.075	0.07	0.04	0.13	0.08	0.09	0.07
209	0.08	0.045	0.095	0.075	0.06	0.078	0.06	0.08	0.09	0.025	0.10	0.15
212	0.08	0.04	0.115	0.09	0.12	0.084	0.04	0.08	0.10	0.03	0.10	0.14
302	0.09	0.09	0.10	0.105	0.06	0.10	0.09	0.10	0.11	0.09	0.11	0.08
405	0.08	0.045	0.08	0.04	0.075	0.075	0.09	0.06	0.09	0.065	0.08	0.15
509	0.11	0.09	0.118	0.105	0.075	0.135	0.12	0.10	0.12	0.11	0.11	0.11
603	0.08	0.10	0.075	0.075	0.105	0.12	0.09	0.07	0.12	0.09	0.10	0.12
612	0.09	0.10	0.08	0.075	0.105	0.11	0.09	0.08	0.14	0.07	0.09	0.12
708	0.10	0.10	0.09	0.09	0.105	0.13	0.09	0.15	0.16	0.11	0.11	0.035
802	0.09	0.05	0.063	0.06	0.075	0.065	0.09	0.12	0.09	0.09	0.075	0.14

\* Prepared with milk held at 143° F. for 30 minutes.

TABLE 3.—Continued

SAMPLE NUMBER	COOPERATING LABORATORIES											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Milk held at 143° F. for 30 minutes plus 0.15% raw milk</i>												
109	0.05	0.05	0.07	0.05		0.052	0.05	0.04	0.07	0.053	0.055	0.04
205	0.055	0.035	0.07	0.05	0.06	0.05	0.04	0.06	0.07	0.015	0.06	0.08
308	0.06	0.04	0.07	0.06	0.05	0.07	0.06	0.06	0.07	0.05	0.06	0.04
401	0.045	0.035	0.055	0.035	0.135	0.05	0.07	0.05	0.05	0.065	0.045	0.08
504	0.06	0.045	0.07	0.06	0.075	0.08	0.08	0.07	0.07	0.07	0.07	0.05
609	0.06	0.06	0.058	0.06	0.075	0.07	0.06	0.06	0.09	0.07	0.06	0.15
703	0.07	0.045	0.053		0.05	0.09	0.06	0.09	0.09	0.08	0.07	0.035
807	0.05	0.025	0.04	0.06	0.05	0.05	0.06	0.06	0.06	0.055	0.06	0.09
<i>Light cream prepared from heavy cream held at 145° F. for 30 minutes plus milk held at 143° F. for 30 minutes plus 0.3% raw milk</i>												
111	0.07	0.10	0.105	0.075		0.075	0.06	0.04	0.11	0.11	0.07	0.09
207	0.09	0.04	0.11	0.075	0.09	0.085	0.06	0.07	0.11	0.02	0.10	0.14
304	0.09	0.06	0.105	0.105	0.075	0.08	0.09	0.09	0.11	0.07	0.09	0.06
403	0.07	0.04	0.07	0.05	0.075	0.07	0.09	0.05	0.10	0.07	0.07	0.11
412	0.07	0.035	0.08	0.105	0.05	0.07	0.07	0.07	0.09	0.06	0.07	0.12
501	0.10	0.10	0.11	0.105	0.075	0.13	0.12	0.12	0.12	0.09	0.10	0.08
606	0.07	0.09	0.075	0.105	0.075	0.125	0.09	0.07	0.12	0.07	0.085	0.09
711	0.10	0.09	0.09	0.09	0.15	0.135	0.12	0.15	0.12	0.10	0.10	0.04
810	0.09	0.05	0.062	0.06	0.075	0.065	0.09	0.09	0.10	0.065	0.085	0.15
812	0.09	0.045	0.06	0.04	0.04	0.065	0.09	0.09	0.08	0.075	0.08	0.12

TABLE 4.—Comparison of color standards for present tentative method

LABORATORY	PHENOL VALUES: MG. PER 0.5 ML. OF MILK SAMPLE								
	.01	.02	.03	.04	.05	.06	.09	.12	.15
Referee	.01	.02	.03	.04	.05	.06	.09	.12	.15
No.									
2	.015	.025	.035	.05	none	.06	.10	.13	.15
3	.01	.015	.025	.04	.05	.06	.09	.11	.15
4	.01	.01	.02	.03	none	.05	.07	.09	.11
5	.01	.02	.025	.03	none	.05	.065	.09	.12
6	.01	.015	.025	.04	none	.06	.09	.11	.15
7	.01	.02	.03	.04	none	.07	.10	.12	.15
8		.02	.03	.055	.05	.065	.12	.12	.15
9	.01	.02	.025	.04	none	.05	.08	.105	.135
10		.02	.035	.045	.055	.065	.10	.12	.15
11	.01	.02	.035	.04	.05	.065	.09	.11	.15
12	.01	.02	.03	.04	.05	.055	.09	.11	.15

143° F. for 30 minutes. In 94 observations of milk that had been heated at 143° F. for 30 minutes, the treatment was designated correctly in all cases. The results indicate that the present tentative method has a pre-

cision and sensitivity equivalent to that reported in the original comparative study (4).

The results of the comparison of the color standards is given in Table 4. There were some discrepancies in the values of the standards used by the several laboratories, although they were probably not great enough to result in any marked variation in the individual observations.

Comparison of domestic filter papers with the Whatman No. 40 used by Kay in the original test and specified for the present tentative method were also made on this occasion in the Associate Referee's laboratory. The Eaton-Dikeman Company papers, New Filt Nos. 1 and 3 (acid-washed), were found to be satisfactory substitutes.

### RECOMMENDATIONS

It is recommended—

(1) That consideration be given to the adoption of the present tentative method for the pasteurization of milk and cream as official.

(2) That the use of the Eaton-Dikeman New Filt papers Nos. 1 and 3 be permitted as an alternative to the use of Whatman No. 40 filter paper in the present tentative method.

### V. FURTHER STUDY OF TECHNIC FOR USE IN THE FIELD

Scharer's field phosphatase test is based on the colorimetric estimation of phenol liberated from disodium phenyl phosphate in 0.5 ml. of milk incubated at 36°–44° C. for 10 minutes. A collaborative study according to an earlier technic was made in 1938 (5). The results indicate that the method used then detected unheated milk or a pasteurized product containing 2 per cent or more of raw milk; in 21 out of 90 observed readings, however, pasteurized milk was not detected, and in only 58 per cent of the 165 samples tested was incomplete heat treatment indicated. The report therefore proposed that further study be made of the technic before it was recommended for routine use. Scharer subsequently modified the method, and favorable published and other reports indicate that it has been found of value in the control of the operation of pasteurizing plants (6). Roger (7) reported, however, that increasing the time of incubation from 10 to 30 minutes gave a greater sensitivity and precision. Thus a second examination seemed warranted. All the following collaborating laboratories had had experience in performing the test.

(1) Bureau of Milk Sanitation, New York State Department of Health, Albany (Mobile Laboratory No. 1), W. D. Tiedeman, Chief.

(2) Geneva, N. Y. City Laboratory, R. S. Breed, Director.

(3) Chemical Laboratory, New York City Department of Health, Jerome Trichter, Acting Director, Bureau of Food and Drugs.

(4) Farm Products Division Laboratory, The Borden Company, Brooklyn, N. Y., A. J. Powers, Director.



(5) Dairymen's League General Laboratory, Syracuse, N. Y., J. L. Hileman, Chief Chemist.

(6) Sheffield Farms Company, Inc., Laboratory, New York City, M. B. Evert.

(7) Department of Dairy Technology, Ohio State University, Columbus, L. H. Burgewald, Associate Professor.

It was considered advisable to have the test done in the laboratory so that on the basis of controlled laboratory use, criteria for the acceptance of the technic under field conditions could be established. Each laboratory was requested to adhere strictly to the latest published technic (3c), and to prepare its own reagents and color standards in accordance with the procedures outlined. At the close of the study all the color standards used were sent to the Associate Referee for comparison with those prepared in his laboratory.

The samples represented milk taken from commercial plants at various holding periods of pasteurization at 143° F.; milk pasteurized at 142° F. for 25 and 30 minutes in the Associate Referee's laboratory; and commercially pasteurized milk or cream to which had been added definite quantities of raw milk. Six series of 12 samples each were examined weekly, beginning June 4, 1940. The samples were packed in sufficient dry ice to provide refrigeration without freezing for 6-10 hours and were shipped for delivery within 24 hours by parcel post, special delivery.

The laboratories reported their findings for each sample as equal to, less than, or greater than, the standards. The results are recorded in Table 5 and Figure 5. They indicate a greater sensitivity and precision in the test than was found in the previous study. In 92 observed readings on pasteurized samples only 12 failed to indicate the treatment correctly. In the detection of samples representing incomplete heat treatment, 40 of 276 observations were incorrect. However, the samples held at 143° F. for 25 minutes were subjected to a long period of preheating in the commercial plant and thus may represent milk heated to the equivalent of 143° F. for 30 minutes. Samples of pasteurized milk and cream to which raw milk had been added in the proportion of 0.15 per cent and 0.3 per cent, respectively, were correctly designated in 116 of 137 observations.

The comparison of the color standards is given in Table 6. Each standard was compared with one of equal value prepared by the Associate Referee. The values of individual standards checked exactly in all but three instances, and in these the slight discrepancies were of no significance.

The findings in this study indicate that the field test made in the laboratory has sensitivity and precision sufficient to warrant its use as a guide in the routine control of pasteurization. Obviously the same degree of accuracy can not be attained in the field where time and temperature of incubation can not be controlled, and where the test is frequently made by a sanitary inspector not skilled in laboratory technic. The test should,



TABLE 5.—Continued

SAMPLE NUMBER	COOPERATING LABORATORIES								TOTAL		
	1	2	3	4	5	6	7	8	a	b	c
<i>Milk held at 142° F. for 30 minutes</i>											
906	a	b	b	b	c	c	b	b	1	5	2
1002	b	b	b	b	c	b	a	b	1	6	1
1108	a	b	c	a	c	c	b	b	2	3	3
1203	a	b	c	b		b		c	1	3	2
1308	b	a	c	b	c	b	a	b	2	4	2
1406	b	b	b	b	c	b	b	b		7	1
Grand total									7	28	11
<i>Light cream prepared from heavy cream held at 145° F. for 30 minutes plus milk held at 143° F. for 15 minutes</i>											
911	b	b	b	b	c	c	b	c		5	3
1006	b	b	b	c	c	b	b	b		6	2
1101	a	b	c	a	c	b	c	b	2	3	3
1207	b	c	c	b		b		c		3	3
1309	b	b	c	a	c	c	b	c	1	3	4
1405	b	b	c	b	b	b	b	c		6	2
1411*	b	b	b	b	c	b	b	c		6	2
Grand total									3	26	17
<i>Light cream prepared from heavy cream held at 145° F. for 30 minutes plus milk held at 143° F. for 30 minutes</i>											
905	b	a	a	a	b	b	a	a	5	3	
1001	a	a	a	a	a	a	a	a	8		
1107	a	a	a	a	a	a	a	a	8		
1202	a	a	b	a	a	a	a	a	5	1	
1306	a	a	a	a	a	c	a	a	7		1
1407	a	a	a	a	a	a	a	a	8		
Grand total									41	4	1
<i>Milk held at 143° F. for 30 minutes plus 0.3% raw milk</i>											
902	a	b	b	b	c	b	b	c	1	5	2
1008	b	a	b	c	c	c	b	b	1	4	3
1103	b	c	c	b	c	b	b	b		5	3
1112*	b	b	c	c	c	c	b	b		4	4
1209	b	b	c	b		c		c		3	3
1305	b	b	c	b	c	b	a	b	1	5	2
1402	b	b	c	b	b	b	b	c		6	2
Grand total									3	28	15
<i>Milk held at 143° F. for 30 minutes plus 0.15% raw milk</i>											
907	a	a	b	b	c	b	a	b	3	4	1
1003	b	a	b	b	c	b	a	b	2	5	1
1109	a	b	b	a	b	b	a	b	3	5	
1204	a	b	b			b		b	1	4	
1301	b	b	b	b	c	a	a	b	2	5	1
1408	b	a	b	b	b	b	b	b	1	7	
Grand total									12	30	3

\* Duplicate sample; results not included in grand total.

TABLE 5.—Continued

SAMPLE NUMBER	COOPERATING LABORATORIES								TOTAL		
	1	2	3	4	5	6	7	8	a	b	c
<i>Light cream prepared from heavy cream held at 145° F. for 30 minutes plus milk held at 143° F. for 30 minutes plus 0.3% raw milk</i>											
910	a	b	b	b	c	c	b	c	1	4	3
912*	c	b	b	b	c	c	b	c		4	4
1011	a	b	b	b	c	b	b	b	1	6	1
1106	a	b	c	b	c	b	b	b	1	5	2
1201	a	b	b	b		b		c	1	4	1
1303	b	b	b	a	c	b	a	b	2	5	1
1312*	b	b	b	a	c	b	a	b	2	5	1
1404	b	b	c	b	b	b	b	c		6	2
Grand total									6	30	10

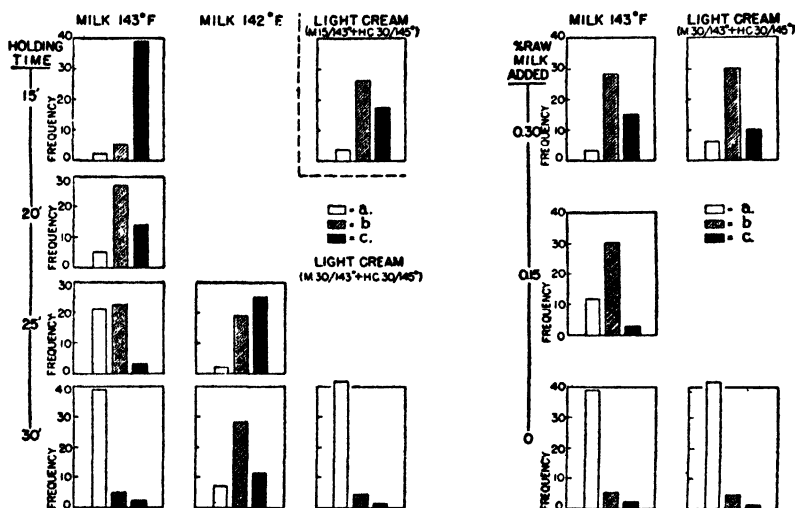


FIG. 5.—FIELD PHOSPHATASE TEST.

Frequency distribution of observed readings by comparison with color standards for milk and cream heated for the given time at the given temperature; values for pasteurized milk and cream with added raw milk. a = < 2 color units; b = ≥ 2, < 5 color units; c = ≥ 5 color units.

therefore, be checked at frequent intervals in comparison with the tentative method of the Association (3a). The field test should not be substituted for the more precise laboratory methods when official results are required.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the field phosphatase test be considered satisfactory for use in the routine control of pasteurization in the field.

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 57 (1941).

TABLE 6.—*Comparison of color standards for field test*  
(Units of color as determined by visual comparison with standard prepared by Associate Referee.)

LABORATORY	COLOR VALUES	
	2 UNITS	5 UNITS
Referee No.	2	5
1	2	5
2	2	5
3	2	5
4	2	5*
5	2	5
6	2†	5†
7	2	5†

\* Slight bluish off-shade.

† Intensity of color slightly less than standard; difference not measurable.

(2) That the field test should not be substituted for more precise laboratory methods when official results are required; and, therefore, that it should not be established as a tentative standard method of the Association.

#### REFERENCES

- (1) SCHARER, H., *J. Dairy Sci.*, 21, 21-34 (1938).
- (2) SCHARER, H., *J. Milk Tech.*, 1, 35-38 (1938).
- (3) AMERICAN PUBLIC HEALTH ASSOCIATION AND ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS, 7TH ED. NEW, YORK, AMERICAN PUBLIC HEALTH ASSOCIATION (1939).
  - a. Method No. II. Tentative Method—Association of Official Agricultural Chemists, 170-174.
  - b. Method No. III. 1. The Rapid Phosphatase Test, 174-178.
  - c. Method No. III. 2. Field Test, 178-179.
- (4) GILCREAS, F. W., *This Journal*, 21, 372-78 (1938).
- (5) —, *Ibid.*, 22, 497-507 (1939).
- (6) BURGWALD, L. H., *J. Dairy Sci.*, 22, 853-873 (1939).
- (7) ROGER, D. M., *Am. J. Pub. Health*, 28, 1325-1327 (1938).

No report on tests for pasteurization of butter was given by the associate referee.

No report on difference between dairy products made from cows' milk and those made from the milk of other animals was given by the associate referee.

## REPORT ON FROZEN DESSERTS

By F. LESLIE HART (U. S. Food and Drug Administration,  
Los Angeles, Calif.), *Associate Referee*

A review of the methods for the analysis of ice cream thus far adopted by this Association reveals that there are official methods for the determination of fat and nitrogen, and a tentative method for the detection of coloring matter. These methods are officially applicable to plain ice cream only, and, through implication, to plain ice cream mix. Much of the Referee's time, in past years, has been spent in unsuccessful attempts to devise a modification of the rapid Babcock method for fat of sufficient accuracy and reproducibility to justify its inclusion as an A.O.A.C. method. The need of such a method still remains, but the recommendation of Referee Frary, *This Journal*, 19, 379 (1936), that further study be discontinued has been confirmed by the Committee on Laboratory Methods, American Public Health Association, A. H. Robertson, Chairman.<sup>1</sup>

It appears that much work needs to be done by this Association on ice cream and similar frozen desserts. As reported by the Referee on Dairy Products last year, *This Journal*, 23, 451 (1940), cooperative arrangements have been made with the Referee on Chemical Analysis of Frozen Dessert Ingredients, American Public Health Association (J. H. Shrader), so as to avoid duplication of effort. In line with this arrangement, the Associate Referee is submitting a method for the determination of solids, and is also reporting limited amounts of work on other determinations, which he hopes will develop to the point where definite methods can be studied by collaborators.

There is great need for a method of preparation of sample applicable to fruit and nut ice creams. A recent method by Maack and Tracy<sup>2</sup> appears worthy of study and it is hoped that a report can be made next year on this subject.

Through private correspondence with Shrader, arrangements were made for the Associate Referee to study methods for the detection of stabilizers in frozen desserts. Some progress has been made, but a method has not been sufficiently developed to warrant submission to collaborators. This study will be continued next year.

## TOTAL SOLIDS

The determination of total solids in most dairy products is complicated by the presence of lactose. In addition, ice cream usually contains dextrose. Both of these sugars crystallize with one molecule of water. Dextrose becomes anhydrous at 110°C. and lactose at 146°C. The question at once arises whether the term "total solids" should be considered, as Frary points out, *This Journal*, 23, 453 (1940), a measure of the total dry solids

<sup>1</sup> *J. Milk Tech.*, 2, 184 (1938).

<sup>2</sup> *Ibid.*, 4, 123 (1940).

content or the solids remaining after heating under definitely prescribed time and temperature conditions. In this connection unpublished work by Wichmann in 1928 is of interest. He determined loss in weight of pure hydrated lactose (5 per cent water of crystallization) when heated under various conditions of pressure and temperature and found that lactose, heated in the air oven at 100°C. loses 0.04 per cent in 4 hours and 0.11 per cent in 5 hours. This he attributes to adhering moisture. On the other hand, when the lactose was heated for 5 hours at 28" vacuum and 100°C., the loss of weight was 3.11 per cent, while in 20 hours it equaled the theoretical value of 5 per cent. This indicates a slow release of the water of crystallization of hydrated lactose under these conditions, but which does not occur at atmospheric pressure and 100°C. to an appreciable extent for at least 5 hours. Preliminary experiments by the Associate Referee indicate that the rate of loss in drying ice cream depends somewhat on the rate of air flow through the vacuum chamber of the drying oven. Robertson reports in a personal communication slightly more solids obtained by drying two samples in a vacuum oven at 100°C. for 3 hours (39.11 and 38.12 per cent) than by drying in an air oven for 4 hours at the same temperature (38.88 and 38.03 per cent). On the other hand, the Associate Referee obtained 37.59 per cent solids on a sample of ice cream dried in an air oven at 100°C. to constant weight (4 hours) and 37.34 per cent by drying to constant weight in a vacuum oven at 100°C. (3½ hours). In this case, dry air was drawn through the oven at a rate of 3-4 bubbles per second. In another determination on the same ice cream, air being admitted at the rate of 1 bubble per second, 37.53 per cent solids were obtained after 4 hours. The figures reported in each case are the average of closely agreeing duplicates.

A series of determinations was then run on a vanilla ice cream under varying conditions as shown in the table. "Constant weight" assumes less than 0.1 per cent loss on further drying for 30 minutes.

TYPE OF OVEN (ALL AT 100°C.)	WEIGHT OF SAMPLE	TIME REQUIRED FOR CONSTANT WEIGHT	SOLIDS
	grams	hours	per cent
Air oven	1.0155	3	37.50
	1.0067	3½	37.44
	1.2840	4	37.40
Same, with dried asbestos in drying dish	1.0777	2½	37.29*
	1.0346	2	37.27
Forced-draft air oven	1.2067	3	37.36
	1.0743	3	37.44
Vacuum oven (air admitted at rate of 1-2 bubbles per second)	1.0528	4	37.40
	1.0870	4	37.45

\* Dried material was slightly darkened.

A similar experiment on chocolate ice cream (work performed by J. F. Armstrong), resulted in the following figures, which are the average of closely agreeing triplicates: Vacuum oven for 3 hours—42.24 per cent, air oven for 4 hours—42.44 per cent, forced draft air oven for 4 hours—42.24 per cent. On the same sample the Associate Referee obtained 42.20 per cent after 4 hours with the vacuum oven, and 42.42 per cent with the air oven for the same time.

A sample of ice cream was then submitted to three collaborators, with instructions to prepare the sample according to *Methods of Analysis*, A.O.A.C., 1940, 306, 132 and to determine total solids (1) in an air oven at 100°C., following the official method, *Ibid.*, 270, 8, but without sand; and (2) in a vacuum oven at 100°C., using only slight vacuum for the first half hour, then increasing the vacuum to a point equivalent to 25 mm. or less of mercury. In both cases a sample of 1-2 grams was specified, and collaborators were instructed to weigh the dishes after 3 hours' drying, and at hourly intervals until constant weight was obtained (loss of 1 mg. or less).

Collaborator:	No. 1	No. 2	No. 3
Solids—Air oven at 100°C.	40.41	40.40	40.45
	40.18	40.33	40.38
Solids—Vacuum oven at 100°C.	40.21	40.28	40.18
	40.24	40.21	40.26

All collaborators reported that constant weight was obtained after 4 hours.

### CONCLUSIONS

It is believed that all official methods for the determination of total solids (or moisture) should specify definite conditions under which the determination is to be conducted. Experiments reported herein show that drying in a vacuum oven at 100°C. gives lower and more erratic results for total solids, presumably through gradual loss of water of crystallization of the hydrated sugars present.

### RECOMMENDATIONS\*

It is recommended—

(1) That the following method for determination of total solids in ice cream be adopted as tentative:

Proceed as directed in XXII, 8, but without sand, using 1-2 gram sample. The sample may be weighed by means of a short, bent, 2 ml. measuring pipet. Consider as total solids the residue obtained after heating for 4 hours.

(2) That study on the detection of stabilizers in ice cream be continued.

(3) That a study on methods for preparation of samples of fruit and nut ice cream for analysis be undertaken.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 56 (1941).



No report on unification of methods for ash in milk and evaporated milk was given by the associate referee.

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No report on sugars in sweetened condensed milk was given by the associate referee.

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No report on naval stores was given by the referee.

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No report on paints, paint materials, and varnishes was given by the referee.

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No report on accelerating testing of paints was given by the associate referee.

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No report on varnishes was given by the associate referee.

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No report on leathers and tanning materials was given by the associate referee.

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## REPORT ON RADIOACTIVITY

By ARTHUR WOLF (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

Exhaustive research in subatomic phenomena in the past decade has had as a corollary extensive improvements in methods of evaluation of radioactivity, and also the development of new methods of measurement. In cases of revision, the fundamental principle has been unchanged but new instruments and apparatus design, as well as improvements in manipulative technic have been applied to these basic principles with gratifying results.

Since there has been no comprehensive report on radioactivity in the past few meetings of this Association, perhaps a brief résumé of the underlying principles involved in radioactivity measurements will be helpful.

All radioactivity phenomena are manifested by the emission, from the nucleus of the atom, of either an alpha particle, a beta particle, or a gamma ray, or as occur in some cases, the simultaneous emission of a gamma ray with either an alpha or a beta particle. These particles and rays exhibit certain properties, among them the ability to ionize gases, and it is upon this property that the system of radioactivity measurements is based.

The relative ability of the alpha, beta, and gamma rays to ionize gases is roughly in the ratio of 10,000:100:1. In other words, under identical conditions an alpha particle produces approximately 10,000 times more

ions than a gamma ray, and therefore evaluation of radium by the alpha particles from radon in equilibrium with its short-lived end products, Radium A, Radium B, and Radium C, is at least 10,000 times more delicate than a gamma ray measurement.

The fundamental principles underlying radium determination by the radon method as developed by Boltwood<sup>1</sup> and improved by Lind<sup>2</sup> and which is the method described in *Methods of Analysis*, A.O.A.C., 1935, has remained unchanged, but critical examination by Evans *et al.*<sup>3</sup> has pointed out serious errors in this older method, and in the past few years these men have developed and improved apparatus design and manipulative technic to such an extent that  $10^{-14}$  grams of radium can be measured with the same ease and rapidity and with greater accuracy than  $10^{-10}$  grams could formerly be measured.

During the past year an apparatus embodying most of these improvements has been set up by the Referee in the Food and Drug Administration's laboratory and has been in use for the past few months. Preliminary results have fulfilled expectations as to the sensitivity of the apparatus. Since a description of the apparatus and the method appears in *Methods of Analysis*, A.O.A.C., 1940, 552, further descriptive detail will be omitted here, but a few of the sources of error which this apparatus eliminates will be briefly enumerated.

(1) Simple boiling of the radium solution, as used in the older apparatus, removes only 90–95% of the radon. Also, the use of atmospheric air to carry the radon to the ionization chamber introduces radioactive contamination, which may sometimes amount to as much as  $1 \times 10^{-12}$  curies of radon. The use of tanked, radioactively inert  $N_2$  for streaming through the boiling solution and as a carrier for the released radon eliminates both of these sources of error.

(2) Radon is strongly adsorbed on organic matter such as rubber and grease. The elimination of the rubber tubing and rubber stoppers of the old apparatus by the use of an all-glass set-up takes care of this source of error.

(3) In the older apparatus, spurious ionization effects occurred because of the inability to completely dry the gas before introduction to the ionization chamber. Provision is now made for complete drying of the gases.

(4) In the new apparatus, the ionization chamber has been made larger, allowing the alpha particles to traverse their complete range. This increases, substantially, the ionization current, and allows more accurate reading.

(5) In the new apparatus accurate control of the charging potential of the electroscope and of the electrode with adequate provision for the control of the electrical state of the measuring instruments eliminates the many sources of error caused by the use of electrically undefined electroscopes and charging devices whose electrical states were unknown.

To summarize, then, the radon method that appears in the new edition of *Methods of Analysis* embodies all the latest improvements in technic and design that have appeared in the literature in the past decade. The sensitivity of the determination is such that, depending upon the delicacy

<sup>1</sup> *Phil. Mag.*, 9, 590 (1905).

<sup>2</sup> *Ind. Eng. Chem.*, 7, 1024 (1915).

<sup>3</sup> Evans, Goodman, Keevil, Lake, and Urry, *Physiol. Rev.*, 35 (May, 1939); Evans, *Rev. Sci. Instruments*, 6 (April, 1935).

of the instrument used for the electrical measurements, the ultimate limit of  $10^{-14}$  grams of radium may be measured.

In gamma ray measurements the advances in methods for evaluation of radioactivity have been even greater than in alpha ray. The filament-type gamma ray electroscope described in *Methods of Analysis, A.O.A.C.*, 1935, has remained unchanged and is still the best instrument for measurement of comparatively large quantities of radium. Below  $10^{-6}$  grams of radium, however, the time required for a reading becomes tediously long and the accuracy of the instrument itself is subject to question. In the past five years, however, a new instrument has been developed for use in beta and gamma ray measurement, the instrument known as the quantum counter. The principle of the instrument is comparatively simple and consists essentially of three parts: (1) the detecting unit known as the Geiger-Müller counter tube; (2) the amplifying and leveling unit; and (3) the recording unit.<sup>4</sup>

In the past year, two of these instruments of different design, both for use in gamma ray measurements, have been in constant operation in the radium laboratory of the Food and Drug Administration. Instrument No. 1 was built by an outside company according to specifications drawn up by the Referee, and instrument No. 2 was built by the Referee according to a design developed in cooperation with the radium laboratory of the Bureau of Standards. Comparison of the two instruments has not been completed, but certain general observations may be pertinent at this time.

(1) With proper care, an instrument of this type may be used for accurate measurements of radioactivity by gamma ray down to  $10^{-8}$  grams of radium and this measurement can be made in less time and with greater accuracy than the measurement of  $10^{-6}$  grams by means of the gamma ray electroscope.

(2) The instrument can be used in place of the gamma ray electroscope.

(3) Because of the design of this type instrument, errors due to secondary effects, such as scattering, size of sample, cosmic radiation, spurious ionization effects, etc., can be more accurately accounted for and corrected than by the use of the electroscope.

By simply substituting a beta ray counter tube for the gamma ray counter tube, this instrument can be used for beta ray analyses.

In the past two years, artificial radioactive isotopes of nearly all the elements have been made, and these provide a new tool in nearly all the sciences; for example in chemistry as an indicator and tracer, in mathematics as a means to study the validity of random distributions, and in biology as a tracer in physiological processes. In fact, the use of the quantum counter for beta ray evaluation promises to become most important since nearly all these artificial radioactive isotopes emit only beta rays.

Because of the extensive use of these instruments for gamma ray measurements, the Bureau of Standards has cooperated with the Referee in making available sets of gamma ray standards to be used with these

<sup>4</sup> L. F. Curtiss, *Nat. Bur. Standards J. Research*, 21 (Dec., 1938); Evans and Alder, *Rev. Sci. Instruments*, 10 (Nov., 1939); Hamblin and Johnson, *Phil. Mag.*, 24, 7 (1937); Oddie, *Proc. Phys. Soc.*, 51, 6 (1939).

counters. These sets, containing 13 standards, are arranged exactly the same as a set of analytical weights ranging in value from 0.1 microgram of radium up to a 100 microgram standard.

As is the case with practically all new developments in the scientific field, extensive work has been done on quantum counting, and the publications on the subject continue to increase, and as would be expected, many contradictions and erroneous conclusions are to be found in this wealth of material. In an attempt to clear up many misunderstandings and to a degree to bring harmony out of chaos, there is now being held in Cambridge, Mass., a colloquium of physicists to discuss these many problems. It is because the Referee is attending this conference that he is unable to be here to present this report. It was considered unwise to submit any new methods for tentative adoption until the results of this Applied Nuclear Physics Conference had been considered.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That the investigation on gamma ray measurements by means of the quantum counter be continued.
- (2) That an investigation be inaugurated on beta ray measurements.

#### REPORT ON CEREALS

By V. E. MUNSEY (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

L. H. Bornmann, Associate Referee on Rye in Flour Mixtures, reported to the Association in 1933, *This Journal*, 17, 405 (1934). On the basis of this report the Committee on Recommendations recommended, *Ibid.*, 58 and 18, 59 (1935), respectively, that the tentative chloroform test for the detection of rye flour in flour mixtures be further studied in connection with the modified Tillmans, *Ibid.*, 17, 405 (1934), and the König-Bartschat<sup>1</sup> methods. The following year, *Ibid.*, 19, 64 (1936), the Committee recommended that the study on the detection of rye in flour mixtures be discontinued. Practically all the studies on the detection of rye have been on flour mixtures and not on the estimation of the amount of rye in rye bread. While this problem may seem an impossibility, it is believed that some study of it should be made. Accordingly, it is recommended that an associate referee be appointed to study the detection and estimation of rye flour in rye bread and to make whatever further studies seem warranted in connection with the Committee's recommendations in *This Journal*, 18, 59 (1935),

No collaborative studies on the analysis of soya flour by the methods for wheat flour have been made by the Association. While soya flour is not a

\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 50 (1941).

<sup>1</sup> *Z. Untersuch. Nahr. Genussm.*, 46, 321 (1923).

cereal, it is frequently used as a cereal and probably could be analyzed by the methods given in the cereal chapter. Accordingly, it is recommended that the present Associate Referee on Soya Flour in Foods include under this subject a collaborative study on the methods for moisture, ash, fat, crude fiber, and protein, similar to the studies reported to the Association in 1939 on oats, corn, rye, and buckwheat products.

The Associate Referee on Phosphated Flour has been inactive for the past two years. It is believed this problem is of sufficient importance to warrant the continuation of the study of the application of the Gustafson method, *Methods of Analysis, A.O.A.C.*, 1940, 212, or any necessary modification for the determination of the original ash in phosphated and self-rising flour. The method for cold water extract in flour has been made official and no further collaborative study seems necessary. Accordingly, it is recommended that this subject be discontinued.

The following recommendations are based on the work of the associate referees.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the colorimetric method for the determination of hydrogen-ion concentration of cereal products with sulfonphthalein as indicator be made official and that further work be done to establish a satisfactory procedure for the determination of hydrogen ion of cereal products by an electro-metric method.

(2) That further study be given to methods for starch in raw and cooked cereals.

(3) That further study be given to fat acidity in grain, flour, corn meal, whole wheat flour, and the correlation of fat acidity with unsoundness of corn meal and whole wheat flour.

(4) That further study be given to the determination of sugar in flour by the method of the associate referee and to its application to the determination of sugar in bread and other cereal products.

(5) That the study of the baking test for soft wheat flour be continued.

(6) That study of the method for the determination of chlorine in the fat of flour be continued.

(7) That the method for the determination of benzoyl peroxide in flour be further studied.

(8) That study of methods for the determination of carotenoid pigments in flour be continued.

(9) That study of methods for the determination of CO<sub>2</sub> in self-rising flour be continued.

(10) That the lactose procedure, with any necessary modifications, *Cereal Chem.*, 13, 541 (1936), for the estimation of milk solids in bread be further studied collaboratively.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 65 (1941).

(11) That study of the method for estimation of butterfat in bread be continued.

(12) That study of proteolytic activity of flour, as recommended by the Associate Referee, be continued.

(13) That study of color measurements of flour and bread be discontinued until methods for measurement are more definitely established.

(14) That study of methods for the determination of cellulose in whole wheat flour products be continued.

(15) That further study be made of the official methods for the determination of moisture, ash, protein, crude fiber, and fat in flour as applied to rye, oats, corn, and buckwheat products.

(16) That the methods for moisture, p. 211, 2, ash, protein, crude fiber, and fat, p. 213, 10, for flour, *Methods of Analysis, A.O.A.C.*, 1940, be adopted as tentative for the analysis of rice and barley products, and that study be continued.

(17) That study be continued on methods for the determination of moisture, ash, protein, fat, and crude fiber in bakery products, including those containing fruits.

(18) That further study be given to the determination of moisture in self-rising flour and pancake, waffle, and doughnut flour.

(19) That studies be continued on methods for the identification of the raw materials used in the manufacture of macaroni.

(20) That the method described by the associate referee for the determination of unsaponifiable matter in noodles and the farinaceous ingredients of noodles be adopted as official, first action.

(21) That the method developed by the associate referee for the determination of sterols in noodles and the farinaceous ingredients of noodles be adopted as official, first action.

(22) That these methods be applied to other farinaceous egg-containing products and further studied collaboratively.

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No report on macaroni products was given by the associate referee.

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## REPORT ON H-ION CONCENTRATION OF CEREAL PRODUCTS

By GEORGE GARNATZ (The Kroger Food Foundation,  
Cincinnati, Ohio), *Associate Referee*

The report of the Committee on Recommendations, *This Journal*, 23, 478 (1940), recommended that the colorimetric method for the determination of the hydrogen-ion concentration of cereal products with sulfonphthalein indicators as outlined, *Ibid.*, 482, be made official, first action. Therefore the work of the Associate Referee was planned to recheck the recommended method to determine if it could be given official status.

TABLE 1.—*Collaborative results*

COLLABORATOR NO.	FLOUR		BREAD		MACARONI		CRACKERS		BISCUIT	
	COLOR.	ELECT.	COLOR.	ELECT.	COLOR.	ELECT.	COLOR.	ELECT.	COLOR.	ELECT.
<i>First Test</i>										
1	A5.2 <sup>1</sup> B5.2	5.12 <sup>2</sup>	B5.6 <sup>1</sup>	5.65	B6.3 <sup>1</sup> C6.3	6.35	C7.3 <sup>1</sup> D7.3	7.30	D8.5 <sup>1</sup>	8.33
2	A5.0 B5.0	5.00 <sup>4</sup>	B5.5	5.50	B6.0	6.00	C7.2 D7.2	7.18	D8.2	8.22
3	5.3 <sup>3</sup>	5.20 <sup>5</sup>	5.7	5.63	6.3	6.17	7.3	7.31	8.4	8.36
4	A5.1 B5.0	—	A5.3 B5.3	—	B5.8	—	C7.2	—	D8.3	—
5 <sup>6</sup>	A5.0 B5.0	4.95 <sup>3</sup>	B5.5	5.50	B6.0 C6.0	5.98	C7.0 D7.0	6.95	D7.8	7.75
6 <sup>6</sup>	A5.0 B5.0	4.93 <sup>3</sup>	A5.1 B5.2	5.35	B5.8	5.80	C7.0 D7.1	6.94	D7.7	7.70
Av.	5.07	5.04	5.40	5.53	6.06	6.06	7.16	7.14	8.15	8.07
Max.	5.3	5.20	5.7	5.65	6.3	6.35	7.0	7.30	8.5	8.33
Min.	5.0	4.93	5.1	5.35	5.8	5.98	7.3	6.95	7.8	7.75
Range	0.3	0.27	0.6	0.30	0.5	0.37	0.3	0.35	0.7	.58
<i>Second Test</i>										
1	A5.1 <sup>1</sup> B5.0	5.10 <sup>3</sup>	B5.5 <sup>1</sup>	5.55	B6.0 <sup>1</sup> C6.0	6.05	C7.1 <sup>1</sup> D7.2	7.13	C8.2 <sup>1</sup> D8.1	8.10
2	A5.0 B5.0	5.00 <sup>4</sup>	B5.5	5.50	B5.9	5.90	C7.1 D7.1	7.18	D7.9	7.95
3	B5.1	5.06 <sup>3</sup>	B5.6	5.52	A5.9	5.88	D7.0	7.06	C6.3 7	6.31 7
4	A4.9 B5.0	—	A5.3 B5.5	—	B5.7	—	C6.8	—	D8.0	—
5	A5.0 B5.0	4.95 <sup>3</sup>	B5.5	5.50	B6.0 C6.0	5.96	C7.0 D7.0	7.00	D7.8	7.85
6	A5.0 B5.0	4.86 <sup>3</sup>	A5.4 B5.4	5.43	B5.8	5.85	C6.8 C6.8	6.82	D7.8	7.82
Av.	5.01	5.00	5.46	5.50	5.91	5.93	6.99	7.04	7.97	7.93
Max.	5.1	5.10	5.6	5.55	6.0	6.05	7.2	7.18	8.2	8.10
Min.	4.9	4.86	5.3	5.43	5.8	5.85	6.8	6.82	7.8	7.82
Range	0.2	0.24	0.3	0.12	0.2	0.20	0.4	0.36	0.4	0.28

<sup>1</sup> Indicator used: A—Bromocresol green.  
B—Chlorophenol red.  
C—Bromothymol blue.  
D—Phenol red.

<sup>2</sup> Indicator not stated.

<sup>3</sup> Coleman 3A—glass electrode.

<sup>4</sup> Beckman

<sup>5</sup> Hydrogen-electrode

<sup>6</sup> Samples run six weeks after other collaborators.

<sup>7</sup> Not included in average because values obviously out of line.

Samples of flour, bread, macaroni, crackers, and biscuits, with the necessary sulfonphthalein indicators and chemicals for preparing the colorimetric standards, were sent to the following collaborators:

- (1) R. A. Barackman, Victor Chemical Works, Chicago, Ill.
- (2) Pearl Brown, Perfection Biscuit Co., Fort Wayne, Ind.
- (3) F. A. Collatz, General Mills, Inc., Minneapolis, Minn.
- (4) W. H. Hanson, Commercial Milling Co., Detroit, Mich.
- (5) H. M. Simmons, Mid-West Laboratories, Inc., Columbus, O.
- (6) J. Ruffley, Jr., Kroger Food Foundation, Cincinnati, O.

The directions given to the collaborators for carrying out each of the two series of tests requested are as follows:

*Buffer Solutions.*—Prepare according to "The Determination of Hydrogen-ions" by W. M. Clark, 3rd Ed., pp. 192-201.

*Colorimetric Standard.*—To 20 ml. of the buffered solution in an ampul  $\frac{3}{4}$ " in diameter, add 0.5 ml. of indicator and seal the ampul. (If ampuls are not available, test tubes of equal bore may be used and the ends closed with cotton or cork stoppers.)

*Method for Measuring pH.*—Use the method described in *This Journal*, 23, 483 (1940), but use gravity rather than suction filtration.

The results obtained by the collaborators on the two tests, reported in Table 1, indicate that those participating in the work arrived at substantially the same results using both the colorimetric and the electrometric methods for measuring pH and that there is good correlation between the results obtained by the different collaborators. In only one instance and on only one sample did the results obtained by a collaborator differ materially from those obtained by the others. A comparison of the results obtained by this group with those of the previous group shows approximately the same degree of correlation on the various samples.

There is again some indication that bromocresol green does not give as good color differentiation as the other indicators used in this work. However, this difference is slight and in the opinion of the Associate Referee it does not justify the elimination of this indicator from the list of those recommended.

As a result of the collaborative work done this year the Associate Referee recommends that the colorimetric method for the determination of the hydrogen-ion concentration of cereal products with sulfonphthalein indicators, as outlined in detail below, be made official. It is also recommended that further work be done to arrive at a satisfactory procedure for the determination of the hydrogen-ion concentration of cereal products by an electrometric method.

#### RECOMMENDED COLORIMETRIC METHOD

##### 1. PREPARATION OF INDICATOR SOLUTION

Grind 1 decigram (0.1 gram) of the dry powder in an agate mortar with the quantities of 0.01 *N* NaOH shown below. (The pH range of the indicator is also listed.)



<i>Indicator</i>	<i>0.01 NaOH</i> <i>ml.</i>	<i>pH Range</i>
Bromocresol green	14.3	3.8-5.4
Chlorophenol red	23.6	4.8-6.4
Bromothymol blue	16.0	6.0-7.6
Phenol red	28.2	6.8-8.4

Dilute the above stock solutions to 250 ml. with distilled water for 0.04% indicator reagent.

## 2. PREPARATION OF STANDARD BUFFER SOLUTION

Prepare according to "The Determination of Hydrogen-ions" by W. M. Clark, 3rd ed., pp. 192-201.

(a) *Colorimetric Standard*.—Place 20 ml. of the buffer solution in ampuls  $\frac{1}{4}$ " in diameter; add 0.5 ml. of indicator solution and seal the ampul.

(b) *Measurement of Hydrogen-ion Concentration*.—To 20 grams of the sample (as received basis; in the case of bread take sample from crumb) add 200 ml. of cool, recently boiled distilled water, and digest at 25° C. for 30 minutes, shaking occasionally during the digestion period. Allow the mixture to stand quietly for 15 minutes and then decant the supernatant liquid through a folded, hardened, dry filter paper. Discard the first 5 ml. to come through, but catch the next 20 ml. in a tube exactly like the tube holding the colorimetric standard. Add 0.5 ml. of the proper indicator and compare the resultant color with the prepared standard to get the pH.

A suitable background such as diffused natural daylight, or that furnished by a daylight type of glass is recommended for making the color comparison. For average conditions, a light intensity of 15-20 microamperes, as registered through a Weston photronic cell, is adequate.

A color comparator of the Walpole type to compensate for color and turbidity of the sample is recommended for making the colorimetric determination. Six deep holes, each large enough to hold one color standard, or sample tube, are bored parallel to one another in pairs. Adjacent pairs are bored as close to one another as possible without breaking through. Perpendicular to the holes and running through each pair are bored smaller holes through which the test tubes may be viewed.

The center pair of test tubes holds the solution to be tested plus the indicator and also a water blank. At either side are placed the standards colored with the indicator and each is backed by a sample of the solution under test. The light is placed on the side of the comparator containing the two controls and water blanks.

The Associate Referee wishes again to express his appreciation for the help and cooperation extended by the collaborators who participated in this investigation. Acknowledgment is also made of the work done by J. Ruffley, Jr. and W. C. Reiman in the conduct of the various tests and in the preparation of this report.

No report on starch in raw and cooked cereals was given by the associate referee.

## REPORT ON FAT ACIDITY IN GRAIN

By LAWRENCE ZELENY (Agricultural Marketing Service, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

The fat-acidity value, defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids from 100 grams of material, calculated on a dry-matter basis, has been shown to be a useful index of deterioration in grain (3, 4, 5, 6) and in flour (2). Procedures for determining this value have been adopted by the Association as tentative methods of analysis (1).

Tests of these methods have resulted in fairly good agreement among most collaborating laboratories; but the agreement has not been sufficiently good, particularly in the case of corn, to justify recommending the methods for adoption as official. Present work, therefore, is concerned primarily with finding the causes and the means of reducing this variability among laboratories.

Samples of corn heretofore distributed to collaborators have consisted partly of individual lots of commercial corn and partly of mixtures of different lots of corn so composited that the desired approximate fat-acidity values of the mixtures were attained. Since the degree of deterioration among the individual kernels in such samples would vary over a relatively wide range, it was thought that sampling errors may have been responsible to a considerable degree for the observed variation among laboratories.

Although it is recognized that any serviceable method for determining fat acidity in grain must be applicable to any mixture of sound and damaged grain, it was thought advisable for the present series of collaborative samples to reduce this one cause of variability to a minimum by securing samples as uniform in composition as possible. For this purpose a 100 pound sack of No. 1 yellow corn of the current (1939) harvest was obtained. This corn had a low moisture content and was practically free from damaged kernels. It had a fat-acidity value of about 14, indicative of sound corn. Cracked corn and foreign material were removed by appropriate sieving, and a portion of the cleaned corn was reserved as one of the required samples.

About 40 pounds of the remaining cleaned corn was placed in a large metal can equipped with a tight cover, and sufficient distilled water was added to increase the moisture content of the corn to 16.5 per cent. The covered can was kept in the laboratory at about 25° C., and the contents were thoroughly mixed each day (except Sundays) to insure, so far as possible, a uniform deterioration of the corn. Small samples were withdrawn from time to time for fat-acidity tests in order to follow the course of the deterioration. Figure 1 shows graphically the increase in fat-acidity values during storage. It is interesting to note the rapid increase in rate of deterioration effected by time. This observation is in agreement with

the common experience in the case of commercially stored grain, that although the initial stages of deterioration may proceed slowly, later stages may proceed at a very rapid rate.

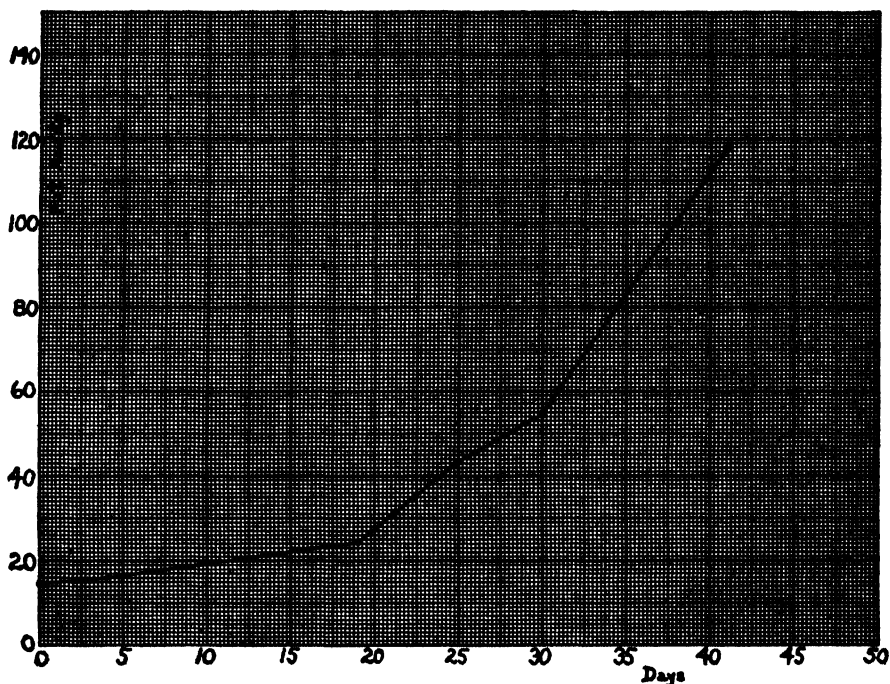


FIG. 1.—SHOWING INCREASE IN FAT ACIDITY OF CORN CONTAINING 16.5 PER CENT MOISTURE THAT WAS STORED IN A METAL CAN AT ABOUT 25° C.

Portions of the corn for the collaborative samples were withdrawn 25 days and 41 days after the adding of the water. These portions were immediately air-dried to about 9 per cent moisture to prevent further deterioration. Both of these portions, as would be expected, were much more uniform in appearance than were samples of commercial corn with corresponding fat-acidity values.

Each of three portions, consisting of the original corn and the corn taken at two different stages of deterioration, was thoroughly mixed and subdivided with the Boerner divider into sub-samples of about 250 grams each to be supplied to the collaborators. The collaborators were instructed to analyze the samples for fat acidity by either the general or the rapid method or by both methods if possible. See *This Journal*, 23, 492 (1940) for details of the methods.

Results were reported by 15 collaborators, 11 of whom reported results obtained by both methods. The data are listed in Table 1. Although

reasonably good agreement is again shown among most of the collaborators, the unexplained variability evident in a number of instances indicates that the search for the causes of these errors must be carried further. The averages show that results obtained by the rapid method tend to be slightly lower than those obtained by the general method. It is believed, however, that this apparent trend is largely accidental since it is due chiefly to the results of a few laboratories and since previous collaborative work, as well as extensive comparison of the two methods by the Associate Referee, shows good agreement between the two methods.

One collaborator (19) suggested the use of alcoholic rather than aqueous standard alkali for the titration as a simpler means of avoiding turbidity in the titration of high acidity samples than that given in the instructions. This would appear to be a useful variation of the method, particularly when large numbers of high acidity samples are being analyzed. It must be remembered, however, that the normality of a standard solution of alcoholic alkali changes rather rapidly and that therefore the solution would require restandardization at frequent intervals.

TABLE 1.—*Results of collaborative study on determination of fat acidity in corn (Set 3)*

LABORATORY	GENERAL METHOD			RAPID METHOD		
	SAMPLE 7	SAMPLE 8	SAMPLE 9	SAMPLE 7	SAMPLE 8	SAMPLE 9
1				39.0	13.0	103.0
2	51.4	21.0	150.0	54.2	18.5	159.4
3				52.7	14.3	133.0
4	51.3	15.2	139.2	54.5	14.7	129.4
5	65.5	21.8	169.0	60.0	21.8	158.0
8	47.0	15.6	129.8	53.0	17.4	127.4
11	44.1	14.4	119.4	43.7	13.2	117.6
12				42.5	12.0	111.0
13				43.5	12.6	113.6
14	52.0	22.0	129.0	46.0	13.0	129.0
15	45.0	16.6	124.5	34.7	12.5	107.2
16	60.9	35.8 <sup>1</sup>	131.8	40.2	16.1	107.4
17	52.6	15.1	123.9	53.8	17.6	118.0
18	50.0	17.3	119.9	49.4	15.4	114.5
19 <sup>2</sup>	51.9	18.2	122.5	50.3	18.5	123.4
Average <sup>3</sup>	52.0	17.7	132.6	47.8	15.4	123.5
Average <sup>4</sup>	52.0	17.7	132.6	49.1	16.2	126.5

<sup>1</sup> Not included in average

<sup>2</sup> Titrations made with 0.0178 N KOH in 95% alcohol.

<sup>3</sup> Includes all laboratories.

<sup>4</sup> Includes only laboratories reporting results obtained by both methods.

The following collaborators participated in this work:

R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill.

F. A. Collatz, General Mills, Inc., Minneapolis, Minn.  
 J. M. Doty, Omaha Grain Exchange, Omaha, Nebr.  
 W. B. Gaessler, Iowa State College, Ames, Iowa  
 A. M. Henry, U. S. Food and Drug Administration, Atlanta, Ga.  
 H. P. Howells, Quaker Oats Co., Cedar Rapids, Iowa.  
 C. W. Ingman, Ingman Laboratories, Minneapolis, Minn.  
 Paul Kolachov, Jos. E. Seagram and Sons, Inc., Louisville, Ky.  
 M. C. Markley, Cargill, Inc., Minneapolis, Minn.  
 B. A. McClellan, General Mills, Inc., Minneapolis, Minn.  
 P. R. Pitts, Evans Milling Co., Indianapolis, Ind.  
 Dorothy B. Scott, U. S. Food and Drug Adm., New York, N. Y.  
 Carl E. Turner, United Mills, Inc., Kansas City, Kans.  
 A. D. Wilhoit, A. D. Wilhoit Laboratory, Minneapolis, Minn.  
 The Associate Referee.

These names are not listed in the order used in the table.

It is recommended that this work be continued in a further effort to obtain greater uniformity in analytical results among different laboratories.

#### LITERATURE CITED

- (1) Report of Subcommittee D., *This Journal*, 22, 68 (1939); 23, 73 (1940).
- (2) ZELNY, LAWRENCE, *Ibid.*, 22, 526-535 (1939).
- (3) —, *Cereal Chem.*, 17, 30-37 (1940).
- (4) —, *This Journal*, 23, 492-496 (1940).
- (5) ZELNY and COLEMAN, *Cereal Chem.*, 15, 580-595 (1938).
- (6) —, U. S. Dept. Agr. Tech. Bull. 644 (1939).

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No report on sugar in flour was given by the associate referee.

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#### REPORT ON BAKING TEST FOR SOFT WHEAT FLOURS\*

By E. G. BAYFIELD (Department of Milling Industry, Kansas State College, Manhattan, Kans.), *Associate Referee*

The following report gives the major results obtained during the 1937-1939 inclusive crop years. Results will be presented and discussed on an annual basis as it seems desirable to treat the material in this chronological fashion. The data should be considered merely as a report of progress. It is hoped that these results may serve as a useful foundation for future work.

Increased interest in soft wheat and soft wheat products during the past few years indicates the need for additional methods for the correct and accurate evaluation of these materials. At present, various workers in the field use several test methods, the method employed frequently

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\* All baking results reported were obtained by the Associate Referee while Cereal Technologist in charge of the Federal Soft Wheat Laboratory, Bureau of Plant Industry, U. S. Dept. of Agriculture, located at and in cooperation with, The Ohio Agricultural Experiment Station, Wooster, Ohio. All bakes were made by C. E. Bode under the direction of the Associate Referee, who desires to express his sincere appreciation for the meticulous care given the work during the course of these investigations.

being based upon the manufactured product. Thus, in the field of cake flours, the worker prefers a test based upon the baked cake, whereas in the field of self-rising soft wheat flours, the chemically-leavened hot biscuit is frequently employed for test purposes. Cookies, pie crusts, the loaf of bread, and other test products might be enumerated.

As a preliminary step in this problem of evolving a satisfactory baking test for soft wheat flours it appeared desirable to obtain the views of workers in the soft wheat flour field as to which test, or tests, they considered most promising. Extensive correspondence and many personal conferences indicated that a bread-baking test might provide a superior means of classifying soft wheat flours over the entire range of flour strength, and also that additional and more specific baking tests might be required after the bread test had properly classified the flour.

It is desirable to avoid unnecessary duplication of work. The American Association of Cereal Chemists has committees actively engaged in the work of evolving satisfactory baking and other tests for cake, self-rising, and soda cracker flours, but has no committee assigned for the formulation of a satisfactory bread-baking test for soft wheat flours although much effort is being expended in this direction upon the hard wheat flours. Therefore work under the A.O.A.C. in the soft wheat field should provide much needed information.

#### PROPOSED METHOD FOR ATTACKING PROBLEM

It is suggested that a suitable baking test be developed along two lines:

(a) That a variety of bread-baking formulas and procedures be tried so that one or two promising methods may be obtained for testing on an expanded basis.

(b) This expanded work shall include the selection of a number of collaborators in various laboratories equipped for, and interested in, a bread test for soft wheat flours.

Correspondence with workers interested in such collaborative studies indicates that the greatest handicap will be a lack of uniformity in available equipment. It is doubtful whether any two laboratories have identical equipment and this is important considering the empirical nature of the baking test. In view of this diversity of available equipment and the variable results from such equipment, no further effort has been made to establish a collaborative committee. However, it is believed that some progress has been made in an effort to find one or more promising baking procedures. This project has been aided by several years of experience in the soft wheat field on the part of the Associate Referee, who has facilities for the work that are modern and that permit all baking to be done in a room with controlled temperature conditions.

#### EQUIPMENT AND METHODS

(1) *Room temperature.*—It was found that a maintained temperature of  $84 \pm 1^\circ \text{F}$ .

for all equipment, flour samples, solutions, etc., produced doughs from the mixer that were close to the desired 86° F.

(2) *Mixer*.—A Hobart C-10 mixer, equipped with Hobart-Swanson attachment (2 pins in bowl), one or two hooks, or cake paddle, as desired, was used. All mixing was carefully timed by means of an electric clock.

(3) *Fermentation cabinet*.—A Bailey cabinet, electrically heated at  $86 \pm 0.5^\circ$  F. was employed. Humidity was maintained at about 92% by means of an electrically operated humidifying unit.

(4) *Oven*.—An electrically controlled and heated Model 150-R Despatch oven equipped with rotating hearth was used. This oven was cased on the bottom and sides with fire brick to provide a steady flow of heat. Moisture in the oven was provided by a beaker containing boiling water, rather than by injected live steam. All bakes were made in 25 minutes at a temperature of  $230 \pm 5^\circ$  C.

(5) *Fermentation bowls*.—Numbered, heavy enamel "oatmeal" bowls, top  $5\frac{1}{2}$ " I.D. and bottom 3" O.D. were used.

(6) *Baking pans*.—Tall-form "pup" loaf pans, *Methods of Analysis, A.O.A.C.*, 1940, 231, constructed of 4XXXX as well as 2XX metal were used. No significant difference between these two types was noted, and therefore the lighter weight metal pan is to be preferred owing to a lower initial cost.

(7) *Loaf volume measuring device*.—Loaves were measured in a device constructed after the hour-glass principle. This device gave direct reading, and it was calibrated by means of standard wooden loaves.

(8) *Supplementary equipment*.—When advisable and convenient to do so, all solutions were dispensed by automatic burets to eliminate possible errors. The yeast solution was kept constantly agitated by means of a small electric stirrer.

#### GENERAL REMARKS PERTAINING TO BAKING METHOD

A basic formula for the experimental test baking of wheat flour is given in the 1940 edition of *Methods of Analysis, A.O.A.C.* This formula is intended for the evaluation of flours whether milled from hard spring, hard winter, soft winter, white, club, or other wheats. Since there is a very wide range in strength and ultimate use of flours milled from these various classes of wheat, it would seem that the same bread-baking test should not be used in their evaluation. Whiting (Blish)<sup>1</sup> and Bayfield and Shiple<sup>2</sup> point out that certain changes in the basic baking formula appear to be necessary for the satisfactory evaluation of soft wheat flours.

In all experiments the flour absorptions were determined for the particular flours concerned, and these pre-determined absorptions were used throughout each experiment. In other words, the absorption was varied to suit the requirements of each flour. Only distilled water was used. In this preliminary work, several factors, namely, sugar concentrations, yeast concentrations, mixing methods, and variations in the malt-phosphate-bromate baking formula were studied. The crumb color is a visual observation, and 100 points are given for optimum whiteness. Less desirable color is indicated in five-point intervals. The letters following the color score are "c" for creamy, "y" for yellow, and "g" for gray, "cy" and "cg" would indicate creamy yellow and creamy gray, respectively. The grain, another visual observation, is given in 5-point intervals, and 100 points

<sup>1</sup> *Cereal Chem.*, 5, 277 (1928).

<sup>2</sup> *Ibid.*, 10, 140 (1933); 14, 551 (1937).

are given for optimum grain. In the data on texture, 100 is equal to a very good texture; 87, to a good; 74, to a fair; 61, to a poor; and 48, to a very poor texture. The loaf type is indicated by a series of letters as recommended by Blish (*loc. cit.*). These loaf types as well as two additional types are also given in a report by Fifield.<sup>3</sup> The optimum crust color is given a value of 10, indicating a brown crust. Darker brown crusts are represented by values of 11 and 12, while lighter crusts are given values between 5 and 10. This same system of recording baking data has been followed throughout this report.

### 1937 CROP SAMPLE EXPERIMENTS

*Baking tests at two sugar levels.*—The results of a study of two sugar levels used with flours from five soft wheat varieties of different strengths are presented in Table 1. The loaf volumes, obtained by the A.O.A.C. basic formula,\* containing 2.5 per cent sugar, are very low, the highest being 398 cc. There is only a range of 66 cc. between the highest and lowest loaf volumes. Also, a poor type of loaf was obtained by this method, and the pale crust color showed evidence of lack of sugar. It is quite apparent that a sugar level of 2.5 per cent is insufficient for the testing of soft wheat flours. When the sugar level was raised to 5 per cent with Formula A, a very noticeable increase in loaf volume was obtained, the highest volume being 590 cc. compared with 398 cc. when 2.5 per cent sugar was used. The range in loaf volume was increased to 97 cc., and there was a general improvement in crumb color, grain, and texture. "M" and "J" represent more superior loaf types than the "H" type loaf obtained in the basic formula. Darker crust colors showed that with this formula there was no lack of sugar. It should also be pointed out that the loaf volumes obtained by Formula A represent more nearly the actual strength of the flours. The behavior of these soft wheat varieties noted in previous work was used in this estimation of flour strength.

*Effect of yeast concentration and mixing times.*—A second factor studied was yeast concentrations, for which a composite of experimentally milled flours was used. The yeast levels employed were 1, 2, 3, and 4 per cent. Another variable, length of mixing time over a very narrow range with the Hobart-Swanson mixer, was introduced with the yeast variable. In these tests 5 per cent sugar and 1 per cent salt were used. As indicated in Table 2, neither the 1 per cent nor the 4 per cent yeast concentration was suitable. The low percentage of yeast gave too low loaf volumes and too dark crust colors. The 4 per cent yeast level, although giving higher loaf

<sup>3</sup> U. S. Dept. Agr. Mimeographed report

\* This formula has been designated as the A.O.A.C. basic formula, *Methods of Analysis, A.O.A.C.*, 1940, page 232, although slight changes have been made in the standard procedure. This procedure states that after the dough has been removed from the mixer, it is folded 20 times in the hands. After mixing, the Associate Referee has merely rounded up the dough before placing it in the fermentation bowl, and also folded it sufficiently to remove the gas (five folds) at the first and second punches. The standard procedure calls for folding 15 times and 10 times, respectively, at the first and second punches. This large amount of handling was frequently found to cause excessive tearing of the dough surface. The other departure from the standard procedure came in the molding method. Canvas belting, rolling pin, and 5/16" wooden strips, as suggested by Merritt, Blish, and Sandstedt,<sup>4</sup> are used. This method has been found superior to the hand-molding procedure.

<sup>4</sup> *Cereal Chem.*, 9, 175 (1932).



TABLE 1.—Comparison of sugar concentrations in baking formula

FORMULA USED	VARIETY	AV. LOAF VOLUME	AV. CRUMB COLOR	AV. GRAIN	AV. TEXTURE	AV. LOAF TYPE AND CRUST COLOR
A.O.A.C. <sup>1</sup> Basic	Purkof	cc. 380	65cy	70	61	H5
	Redrock	366	75cy	65	61	H5
	Nittany	332	65cg	60	48	H5
	Trumbull	398	65cy	65	55	H6
	Am. Banner	365	63cy	58	48	H5
A <sup>2</sup>	Purkof	590	65cy	83	87	M8
	Redrock	560	78cy	75	74	M9
	Nittany	574	73cy	70	74	M9
	Trumbull	547	68cy	75	74	M10
	Am. Banner	493	65cy	63	48	J8

Procedure in both bakes given above:

1. Mixing in Hobart-Swanson mixer 1.5 min.
2. Absorption variable.
3. Fermentation 3 hrs. at 30°C.

<sup>1</sup> A.O.A.C. basic formula contains 2.5 % sugar, 1 % salt, 3 % yeast.

<sup>2</sup> Formula A contains 5 % sugar, 1 % salt, 3 % yeast.

TABLE 2.—Study of yeast concentrations and mixing time

YEAST CONCENTRATION	MIXING TIME	AV. LOAF VOLUME	AV. CRUMB COLOR	AV. GRAIN	AV. TEXTURE	AV. LOAF TYPE AND CRUST COLOR
per cent	minutes	cc.				
1	0.5	438	68c	78	74	M12
	1.0	440	68c	80	74	M12
	1.5	457	71c	78	74	M12
2	0.5	458	63c	70	74	M10
	1.0	462	66c	73	74	M10
	1.5	474	66c	73	74	M10
3	0.5	462	63c	68	74	M10
	1.0	472	63c	68	74	M10
	1.5	485	63c	73	74	M10
4	0.5	483	58c	63	68	M8
	1.0	480	61c	58	68	M8
	1.5	489	63c	61	68	M8

Details applying to formula used above:

1. Flour, 1936 soft wheat flour composite.
2. Mixing in Hobart-Swanson mixer, variable time.
3. 5 % sugar, 1 % salt used.
4. Fermentation 3 hrs. at 30°C.

volumes, was too high a concentration, as shown by the poorer internal scores and lighter crust color. Since a rather vigorous fermentation as a means of flour differentiation seemed advisable, the 3 per cent yeast concentration was adopted. This factor, together with 1 per cent of salt, a 3 hour fermentation at 30°C., 55 minute proof at 30°C., and baking at 230°C. for 25 minutes, was therefore kept as stated in the basic formula. Although the mixing time with the Hobart-Swanson mixer was only varied from a half to one and a half minutes, the longer mixing time seemed desirable.

*Mixing methods.*—Additional data on mixing times and mixing methods are given in Table 3. The soft wheat flour composite used was the same as that giving the data presented in Table 2. Mixing studies were conducted with the Hobart-Swanson mixer, and the mixing time was varied from 0.5 to 4 minutes. Baking data are also presented for doughs mixed with the Hobart mixer equipped with two dough arms, one dough arm, and cake paddle. Examination of these baking data of doughs mixed with the Hobart-Swanson mixer indicates that with this particular flour 2 minutes is the optimum mixing time. Since the A.O.A.C. basic formula stipulates a 1 minute mix, and since the flour used in this experiment was a composite of only the harder soft wheat varieties, a mixing time of 1.5 minutes with the official Hobart-Swanson mixer was decided upon. Additional data are also given in Table 3 to show that if the official mixer is not available, satisfactory mixing can be obtained with the Hobart mixer. From a study of the baking data of mixing obtained with this mixer equipped with two hooks (arms), one dough hook, or the cake paddle, it was concluded that the cake paddle gave more satisfactory results than did either the single or 2-hook mixings.

*Malt-phosphate-bromate formula.*—A fourth study relates to variations in the malt-phosphate-bromate baking formula. Bayfield and Shiple, *loc. cit.*, concluded that such a formula would evaluate soft wheat varieties in a satisfactory manner. It is understood that this formula has been extensively used on spring wheat flours by Canadian workers since the original work by Aitkin and Geddes.<sup>5</sup> In the present study numerous variations of this malt-phosphate-bromate formula were used. The baking data are given in Table 4. Formula 6 in this table had the amounts recommended by Aitkin and Geddes, *Ibid.* In the bake employing 0.001 gram of potassium bromate, the loaf volume shows a negative response when compared with the regular or control bake. The composite flour used gave more satisfactory baking results when only 0.0005 gram of potassium bromate was added per loaf. This is also brought out in further variations of the malt-phosphate-bromate formula. To summarize briefly the data in Table 4, 0.15 gram of diastatic malt (approximately 200° Lintner), 0.05 gram of ammonium phosphate (dihydrogen) and 0.0005 gram of potassium bromate per loaf added to the regular formula gave more satisfactory re-

<sup>5</sup> *Cereal Chem.*, 11, 487 (1934).

TABLE 3.—*Comparison of mixing times and mixing methods*

MIXER	MIXING TIME	AV. LOAF VOLUME	AV. CRUMB COLOR	AV. GRAIN	AV. TEXTURE	AV. LOAF TYPE AND CRUST COLOR
	<i>minutes and mixing speed</i>	<i>cc.</i>				
Hobart-Swanson	0.5	462	63c	68	74	M10
	1	472	63c	68	74	M10
	1.5	483	64c	71	74	M10
	2	505	73c	75	87	M10
	3	493	75c	75	94	M10
	4	496	75c	80	94	M10
Hobart 2 Dough Arms	0.5 low 3 high	489	73c	72	74	M10
	0.5 low 4 high	456	70c	73	81	M10
	0.5 low 5 high	477	71c	71	74	M10
	0.5 low 3 med.	474	68c	70	74	M10
	0.5 low 4 med.	476	73c	73	81	M10
	0.5 low 5 med.	479	72c	72	78	M10
	1 Dough Arm					
	0.5 low 3 high	464	65c	70	74	M10
	0.5 low 5 high	464	75c	70	74	M10
Cake Paddle	0.5 low 2½ high	507	73c	75	87	M10
	0.5 low 3 high	488	73c	73	83	M10
	0.5 low 4 high	455	75c	78	87	M10
	0.5 low 5 high	466	78c	75	74	M10
	0.5 low 2½ med.	475	70c	73	74	M10
	0.5 low 3 med.	479	70c	73	87	M10
	0.5 low 4 med.	487	70c	75	81	M10
	0.5 low 5 med.	485	70c	72	83	M10
	0.75 low 0.5 med.	453	67c	67	74	M9
	0.75 low 0.75 med.	482	70c	73	87	M8

Details of formula used above:

1. Flour, 1936 soft wheat flour composite.
2. 5% sugar, 1% salt, 3% yeast used.
3. Fermentation, 3 hrs. at 30°C.

sults than when the full (double these quantities) amount of malt, phosphate, and bromate were used. If a formula of this nature is to be used in evaluating soft wheat flours, the amounts of malt, phosphate, and bromate used apparently should be less than those used for stronger bread flours.

*Comparison of several baking formulas.*—After concluding the previous work on methods, the Associate Referee considered it to be desirable to test a few possible baking formulas. In Table 5 various baking formulas are compared with the A.O.A.C. basic method. Previous experience with these soft wheat varieties, as well as a knowledge of their behavior com-

TABLE 4.—*Variations in malt-phosphate-bromate bake*

FORMULA	AV. LOAF VOLUME	AV. CRUMBS COLOR	AV. GRAIN	AV. TEXTURE	AV. LOAF TYPE AND CRUST COLOR
	cc.				
(1) Regular	497	68c	70	74	M10
(2) 1 mg. bromate	472	76c	74	87	M10
(3) 0.5 mg. bromate	493	77c	77	83	M11
(4) 0.3 mg. malt	583	68c	75	81	L12
(5) 0.15 gram malt	543	73c	75	81	M12
(6) Malt-phosphate-bromate	522	70c	75	87	M7
(7) $\frac{1}{2}$ malt, phosphate-bromate	510	70c	78	94	M10
(8) $\frac{1}{2}$ bromate, malt-phosphate	546	73c	80	96	M7
(9) $\frac{1}{2}$ phosphate, malt-bromate	501	72c	80	96	M9
(10) $\frac{1}{2}$ malt, $\frac{1}{2}$ bromate, phosphate	528	77c	77	96	M9
(11) $\frac{1}{2}$ malt-phosphate-bromate	549	78c	80	96	M10

Details applying to formulas used above:

Flour, 1936 soft wheat flour composite.

Mixing, in Hobart-Swanson mixer 1.5 min.

Fermentation, 3 hours at 30°C.

(1) Regular method, 5% sugar, 1% salt, 3% yeast.

(2) as in (1) but with 0.001 gram of  $\text{KBrO}_3$  added per loaf.

(3) as in (1) but with 0.0005 gram of  $\text{KBrO}_3$  added per loaf.

(4) as in (1) but with 0.3 gram of diastatic malt (approx. 200°L.) added per loaf.

(5) as in (1) but with 0.15 gram of diastatic malt added per loaf.

(6) as in (1) but with 0.3 gram of diastatic malt, 0.1 gram of  $\text{NH}_4\text{H}_2\text{PO}_4$ , and 0.001 gram of  $\text{KBrO}_3$  added per loaf.

(7) as in (6) but with only 0.15 gram of diastatic malt.

(8) as in (6) but with only 0.0005 gram of  $\text{KBrO}_3$ .

(9) as in (6) but with only 0.05  $\text{NH}_4\text{H}_2\text{PO}_4$ .

(10) as in (6) but with only 0.15 gram of diastatic malt and 0.0005 gram of  $\text{KBrO}_3$ .

(11) as in (1) but with 0.15 gram of diastatic malt, 0.05 gram of  $\text{NH}_4\text{H}_2\text{P}_4$ , and 0.0005 gram of  $\text{KBrO}_3$ .

mercially, indicated that the loaf volumes obtained by Formula B should give the most satisfactory evaluation of flour strength. It is evident from Table 5 that various baking methods rank wheat flours differently. This is not an unexpected result by any means. The important point is to use a method that ranks the flours correctly, and as for a criterion of "correctness" it appears that behavior in commercial practice preferably should be selected. With Formulas A, B, and C, the variety Purkof gives the highest loaf volumes; when the yeast and salt concentrations are varied, as in Formulas D, E, F, and G, Trumbull gives greater loaf volume than does Purkof. Further work must be done to account for these differences.

TABLE 5.—*Comparison of baking formulas for soft wheat flours*

VARIETY	A.O.A.C. BASIC	FORMULA USED						
		A	B	C	D	E	F	G
Loaf Volume (cc.)								
Purkof	380	590	625	710	520	600	520	625
Redrock	366	560	583	668	518	615	525	625
Nittany	332	574	559	693	533	600	505	620
Trumbull	398	547	558	658	540	660	525	650
Am. Banner	365	493	530	584	520	565	485	550
Crumb Color								
Purkof	65cy	65cy	70cy	75cy	70cy	73cy	75cy	90c
Redrock	75cy	78cy	80c	88c	78cy	85c	83c	95c
Nittany	65cg	73cy	70cy	78c	70cy	85c	73cg	85c
Trumbull	65cy	68cy	73cy	77cy	78cy	85c	78cy	85c
Am. Banner	63cy	65cy	68cy	70cy	68cy	78cy	73cy	78cy
Grain								
Purkof	70	83	90	95	90	98	100	100
Redrock	65	75	88	95	88	93	93	100
Nittany	60	70	78	88	78	88	80	93
Trumbull	65	75	88	88	93	95	93	103
Am. Banner	58	63	70	73	73	85	83	83
Texture								
Purkof	61	87	100	100	100	100	100	100
Redrock	61	74	100	100	94	100	94	100
Nittany	48	74	87	94	74	94	74	100
Trumbull	55	74	100	100	94	100	100	100
Am. Banner	48	48	68	74	68	87	74	87
Loaf Type and Crust Color								
Purkof	H5	M8	L9	F12	M10	L12	M9	L11
Redrock	H5	M9	M8	L12	M9	L12	M10	L12
Nittany	H5	M9	M9	F12	M10	M12	M10	L12
Trumbull	H6	M10	MH10	L11	M12	F12	M9	F12
Am. Banner	H5	J8	M8	M10	M9	M13	M8	M11

Procedure applying to all bakes described above:

1. Mixing in Hobart-Swanson mixer 1.5 min.
2. Absorption variable.
3. Fermentation.

	<i>Min.</i>
First punch after	105
Second punch after additional	50
Mold after additional	25
Total fermentation at 30°C.	180
Proof at 30°C.	55
Bake at 230°C.	25

A.O.A.C. basic formula contains 2.5 % sugar, 1 % salt, 3 % yeast.

Formula A contains 5 % sugar, 1 % salt, 3 % yeast.

Formula B contains 5 % sugar, 1 % salt, 3 % yeast, 0.15 gram of 200°L. diastatic malt, 0.05 gram of  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.0005 gram of  $\text{KBrO}_3$ .

Formula C contains 5 % sugar, 1 % salt, 3 % yeast, 0.15 gram of 200°L. diastatic malt, 0.05 gram of  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.0005 gram of  $\text{KBrO}_3$ , 4 % of dried skim milk, 3 % of shortening.

Formula D contains 5 % sugar, 1.5 % salt, 2 % yeast.

Formula E contains 5 % sugar, 1.5 % salt, 2 % yeast, 4 % dried skim milk, 3 % shortening.

Formula F contains 5 % sugar, 1.5 % salt, 2 % yeast, 1 mg. bromate.

Formula G contains 5 % sugar, 1.5 % salt, 2 % yeast, 4 % dried skim milk, 3 % shortening, 1 mg. bromate.

*Summary of 1937 crop data.*—During the first year of work only a start was made in attacking the problems, and it is realized that much of the work done will require repetition to prove its reliability.

Additional studies are required to determine whether less time than 1½ minutes for mixing may be used with the Hobart-Swanson attachment. It seems advantageous to deviate as little as possible from the present basic procedure recommended for hard wheat flours. Information is required regarding the possible effects of variations in fermentation times and temperatures, but here again it seems advisable to adhere to the basic method recommendations except for special tests.

From this year's work it would appear that the use of 5 instead of 2.5 per cent of sugar in the formula is warranted.

#### 1938 CROP SAMPLE EXPERIMENTS

During this year a number of experiments, initially made during the preceding year, were repeated. In addition, some other technological points were investigated before a number of the different formulas were tried out.

*Sugar concentrations.*—It was indicated previously that 5 per cent of sugar is preferred to the 2.5 per cent stipulated in *Methods of Analysis*, A.O.A.C., 1940. By way of confirmation bakes were made with increments of sugar varying from 2.5 to 10.0 per cent. In addition some bakes were made using 6.25 per cent sugar.

As shown in Table 6, loaves of small volume and very pale crust color were obtained from the use of 2.5 per cent sugar; with additional sugar

the loaf volumes varied directly with the sugar level. The data in Table 6 show that crumb color, grain, and texture were improved by increases in sugar level. It was considered that the 7.5 and 10.0 per cent sugar levels were excessive since they gave crust colors that were very dark. The 6.25 per cent level gave larger loaf volumes and superior internal scores as compared to the 5 per cent level. However, the crust colors were still ex-

TABLE 6.—Comparison of various sugar and salt concentrations and mixing 1 or 1½ minutes in the Hobart-Swanson mizer<sup>1</sup>

	1 MIN. MIX					1½ MIN. MIX					
	AV. LOAF VOL- UME	CRUMB COLOR	AV. GRAIN	AV. TEX- TURE	AV. LOAF TYPE AND CRUST COLOR	AV. LOAF VOL- UME	CRUMB COLOR	AV. GRAIN	AV. TEX- TURE	AV. LOAF TYPE AND CRUST COLOR	
	ml.					cc.					
Variable sugar concentrations with 1 % salt, 3 % yeast											
Sugar (%)	481	70cy	74	68	M6	493	71cy	74	71	M6	
2.5	547	70cy	74	74	M10	574	74cy	79	81	M10	
5.0	571	75cy	75	87	L12	575	78cy	80	87	L12	
6.2	621	74cy	76	84	L12	629	76cy	80	87	L12	
7.5	632	73cy	80	81	L13	675	79c	81	84	F13	
10.0	Average	570.3	71.8	76.0	76.8	10.3	592.8	75.0	78.5	80.8	10.3
Variable salt concentrations with 6½ % sugar, 3 % yeast											
Salt (%)	571	75cy	75	87	L12	575	78cy	80	87	L12	
1.0	569	76cy	79	87	M12	587	76cy	83	87	L12	
1.5	Average	570	76cy	77	87	12	581	77cy	82	87	12
Variable salt concentrations with 5 % sugar, 3 % yeast											
0.5	560	70cy	73	74	M8	568	70cy	74	74	M8	
1.0	547	70cy	74	74	M10	574	74cy	79	81	M10	
1.5	554	71cy	80	84	M10	572	75cy	83	87	M10	
2.0	560	75cy	84	87	M10	557	79c	88	90	M10	
2.5	567	76cy	84	98	M12	563	78cy	88	100	M12	
Average	557.6	72.4	79.0	83.4	10.0	566.8	75.2	82.4	86.4	10.0	
3.0	549	76cy	89	100	M11	570	79cy	95	100	M12	
4.0	543	74cy	85	98	M11	557	76cy	93	98	M11	
Average	554.3	73.1	81.3	87.9	10.3	565.9	75.9	85.7	90.0	10.4	
Variable salt concentrations with 6 % sugar, 2 % yeast											
0.5	550	75cy	76	77	M10	560	75cy	76	74	M10	
1.0	549	75cy	80	81	M11	565	75cy	83	84	M11	
1.5	553	75cy	84	84	M12	547	78cy	89	94	M12	
2.0	534	75cy	88	94	M12	537	78cy	93	98	M12	
2.5	524	78cy	95	100	M12	535	79cy	94	98	M12	
Average	542.0	75.6	84.6	87.2	11.4	548.8	77.0	87.0	89.6	11.4	

<sup>1</sup> Commercially milled (V-108) 95% straight grade unbleached flour.

cessively dark. Of the different sugar levels used the 5 and 6.25 percentages were the best. These two sugar levels were also used in studies where the salt concentrations were varied, but there was no indication that the 6.25 per cent sugar level was outstandingly superior to the lower percentage of sugar, which accordingly was used in most of the subsequent work.

*Salt concentrations.*—Table 6 also shows the effect of the various salt concentrations when studied with 5.0 per cent of sugar and both 2.0 and 3.0 per cent of yeast. The decrease in loaf volume and increase in the internal scores is much more pronounced in the 2 per cent yeast level. This would be expected because the fermentation is retarded by the presence of excess quantities of salt and limited quantities of yeast. Bread baked with 3.0 or 4.0 per cent of salt is impractical, not only because of the smaller loaf volume, but also because of the taste of the finished product. If the baking data from the salt concentrations between 0.5 and 2.5 per cent are considered there seems to be little reason why the present quantity of salt, namely, one per cent, should be changed. Unless otherwise stated 1.0 per cent of salt was used in the baking tests.

*Yeast concentrations.*—The matter of yeast concentrations was investigated quite extensively last year when concentrations of from 1.0 to 4.0 per cent were used. In the present studies the yeast was restricted to two levels, namely 2.0 and 3.0 per cent. The results, shown in Table 6, indicate but little difference between the two concentrations. Therefore the higher (3.0 per cent) level was used in the majority of later studies. A contributing reason for this decision was the fact that it was considered that a slight (possible) excess amount of yeast would be an added factor of safety in cases where batches of yeast of reduced activity might inadvertently be used. An excess amount of yeast should aid in preventing this factor from becoming a limiting one.

*Mixing studies.*—Periods of from 0.5 to 2.0 minutes were used with the Hobart-Swanson mixer. These data (Table 7) indicate that mixing for 1.5 minutes in the Hobart-Swanson mixer was the optimum time for the flour used. Shellenberger<sup>6</sup> concluded: "If the properties of the flour will permit, it is advantageous to mix a dough in the Hobart-Swanson mixer for longer than one minute to completely incorporate the ingredients." In order to determine whether a very weak flour would withstand mixing periods up to two minutes in the Hobart-Swanson mixer, an experiment was tried with a bleached commercial cake flour. The results of this test (Table 8) indicate that such a weak flour could withstand two minutes' mixing. Therefore, in order to obtain complete incorporation of ingredients and yet cause as little gluten development as possible, a mixing period of 1.5 minutes in the Hobart-Swanson mixer was chosen for the subsequent work. Additional mixing studies (Table 7) on the Hobart mixer equipped with a 3 quart bowl and either 2 dough arms or a cake paddle show that

<sup>6</sup> *Cereal Chem.*, 15, 197 (1938).



either one of these attachments can be used satisfactorily if the official mixer is not available. Mixing with the cake paddle 0.5 minute on low speed and 2 minutes on high speed gave better results than any obtained with the two-dough arms.

*Use of sheeting rolls for punching and molding.*—Moderately priced sheeting rolls have recently become available on the market and have met with considerable approval in the hands of hard wheat flour chemists. It seemed advisable, therefore, to determine whether the use of these rolls would aid in reducing variability in a soft wheat baking technic. Should this prove to be the case then the purchase of this equipment would be justifiable.

The roll settings on this "sheeter" can be varied from  $1/8$  to  $5/16$  inch. Preliminary experiments showed that a roll setting of  $5/16$  inch gave satisfactory results, and this width was adopted as standard for both the machine punching and molding operations.

The hand punching consists of folding the dough sufficiently to remove the gas, namely five folds at the first and second punches. The hand molding consists of rolling out the dough on a piece of canvas belting, by means of a rolling pin running on wooden strips  $5/16$  inch thick. The dough is then turned over, the outer edges are overlapped, and the dough is again flattened with the rolling pin. The dough is again turned over but with the seam running away from the operator, and the flattened dough is rolled up from the remote end. The seam and ends are sealed by pinching, and the dough is rolled lightly under the palm of the hand to adjust it to the length of the pan.

In machine punching, the dough is removed from the fermentation bowl, and the edges are brought together in order to cover the moist bottom surface; the dough is then run through the sheeting rolls set at  $5/16$  inch. The ends of the resulting dough strip are overlapped, and the dough is returned to the fermentation bowl, seam down. In the machine molding, the dough is again run through the sheeting rolls at  $5/16$  inch. With the resultant strip on a piece of canvas belting, the dough is rolled up by hand from the more remote end. The seam is sealed by pinching, but the ends of the molded dough are left unsealed.

A composite of soft wheat flour residues was used in a study of the various combinations of hand and machine punching and molding. A summary of this baking work is given in Table 9. In examining the data covering the work on hand versus machine punching and molding, the reader should keep in mind that it was done before any definite technic in the use of the sheeting rolls had been established. While the average loaf volumes for Formula B indicate that the machine treatment is more severe than the hand treatment, those for Formula C did not show the same tendencies. Again, in Table 10, Formula B gives a lower average loaf volume with machine punching and molding, whereas Formula C gives a

TABLE 7.—*Mixing experiments (flour V-108)*

MIXER	AV. LOAF VOLUME	AV. CRUMB COLOR	AV. GRAIN	AV. TEXTURE	AV. LOAF TYPE AND CRUST COLOR
<i>Hobart Swanson</i>	cc.				
0.5 min.	561	73cy	71	74	M10
1.0 min.	584	73cy	75	81	M10
1.5 min.	593	74cy	78	81	M11*
2.0 min.	591	75cy	79	84	M10
<i>2 Dough Arms</i>					
0.5 low 1 min. high	575	74cy	76	74	M10
0.5 low 2 min. high	575	75cy	78	81	M10*
0.5 low 3 min. high	542	75cy	79	87	M10
0.5 low 1 min. med.	553	74cy	75	81	M10
0.5 low 2 min. med.	571	75cy	81	87	L11*
0.5 low 3 min. med.	562	76cy	78	84	M10
<i>Cake Paddle</i>					
0.5 low 1 min. high	598	75cy	79	81	M11
0.5 low 2 min. high	595	76cy	81	84	L11*
0.5 low 3 min. high	588	76cy	79	84	M10
0.5 low 1 min. med.	571	74cy	76	77	M10
0.5 low 2 min. med.	589	75cy	79	77	M10
0.5 low 3 min. med.	594	75cy	78	77	M10*

\* Optimum.

TABLE 8.—*Mixing experiments with a cake flour<sup>1</sup>*

MIXER	AV. LOAF VOLUME	AV. CRUMB COLOR	AV. GRAIN	AV. TEXTURE	AV. LOAF TYPE AND CRUST COLOR
<i>Absorption 54.1%</i>					
<i>Hobart-Swanson</i>	cc.				
0.5 min.	281	100w	50	48	H5
1.0 min.	288	100w	50	48	H5
1.5 min.	297	100w	54	48	H5
2.0 min.	304	100w	59	48	H5
<i>Absorption 56.6%</i>					
<i>Hobart-Swanson</i>					
0.5 min.	301	100w	53	48	H5
1.0 min.	312	100w	55	48	H6
1.5 min.	316	100w	56	48	H6
2.0 min.	321	100w	59	48	H6

<sup>1</sup> Commercial cake flour (V-93B), bleached.

TABLE 9.—Loaf volumes from combinations of hand and machine punching and molding

FORMULA B					FORMULA C			
	HAND PUNCHED AND MOLDED	HAND PUNCHED, MACHINE MOLDED	MACHINE PUNCHED, HAND MOLDED	MACHINE PUNCHED AND MOLDED	HAND PUNCHED AND MOLDED	HAND PUNCHED, MACHINE MOLDED	MACHINE PUNCHED, HAND MOLDED	MACHINE PUNCHED AND MOLDED
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
	588	537	537	543	518	527	532	532
	582	527	537	523	502	475	481	518
	537	537	537	475	513	465	523	537
	610	549	582	527	518	465	527	527
	549	527	527	497				
	543	537	549	513				
	—	537	555	560				
	—	537	582	532				
Average	568.2	536.0	550.8	521.3	512.8	483.0	515.8	528.5
Maximum	610	549	582	560	518	527	532	537
Minimum	537	527	527	475	502	465	481	518
Range	73	22	55	85	16	62	51	19

TABLE 10.—Loaf volumes in hand versus machine punching and molding

	FORMULA B		FORMULA C	
	HAND	MACHINE	HAND	MACHINE
	cc.	cc.	cc.	cc.
	634	634	707	687
	652	616	670	720
	687	616	687	720
	658	605	749	767
	676	634	749	749
	610	628	693	736
	687	640	700	749
	676	652	693	786
	628	658	725	743
	682	664	676	749
Average	659.0	634.7	704.9	740.6
Maximum	687	664	749	786
Minimum	610	605	670	687
Range	77	59	79	99

higher average loaf volume by machine but the range in loaf volume between replicates is greater than in any of the other cases.

Table 11 presents another variability study in which machine punching and molding were used. Here again the range in loaf volume from the replicated bakes was very large. These results indicate that the sheeting

TABLE 11.—*Loaf volumes in two baking formulas using machine punching and molding*

	FORMULA B	FORMULA C
	cc.	cc.
	664	693
	664	714
	628	714
	628	687
	640	707
	670	725
	658	714
	664	687
	652	682
	594	693
	652	736
	687	800
	646	720
	664	749
	670	720
Average	652.1	716.1
Maximum	687	800
Minimum	594	682
Range	93	118

TABLE 12.—*Wheat and flour analysis of samples used in 1938 baking formula studies*

NO.	VARIETY	WHEAT DATA						
		FLOUR YIELD	TEST WEIGHT	PROTEIN	MOIS- TURE	ASH	TIME TEST	PEARLING TEST
3752	Purkof	per cent 66.6	pounds 58.9	per cent 9.0	per cent 11.5	per cent 1.627	minutes 77	grams 12.5
3753	Redrock	67.5	58.7	9.7	11.6	1.659	46	10.5
3754	Nittany	73.9	58.2	9.3	11.2	1.682	42	7.4
3755	Trumbull	74.1	59.4	9.8	11.1	1.678	41	8.6
3756	Am. Banner	72.7	57.2	8.5	11.2	1.636	28	7.6

FLOUR DATA

NO.	VISCOSITY		PROTEIN	ASH	MOISTURE	ABSORPTION
	20.0 GRAMS FLOUR	2.0 GRAMS PROTEIN				
	°MacM.	°MacM.	per cent	per cent	per cent	per cent
3752	89	166	7.7	.405	15.0	58.0
3753	86	147	8.0	.399	14.5	53.8
3754	67	116	7.9	.395	14.2	53.9
3755	87	126	8.4	.399	14.1	54.2
3756	28	69	7.1	.395	13.8	48.6

TABLE 13.—*Baking formulas used with 1938 soft wheat flours*

NO.	VARIETY	FORMULA						
		A <sup>1</sup>	A <sup>2</sup>	B <sup>1</sup>	B <sup>2</sup>	C <sup>1</sup>	C <sup>2</sup>	D <sup>2</sup>
Loaf volume (cc.)								
3752	Purkof	428	440	500	510	481	495	425
3753	Redrock	405	428	536	504	487	487	463
3754	Nittany	314	336	492	470	481	479	421
3755	Trumbull	340	359	530	502	525	497	453
3756	Am. Banner	366	371	458	417	433	419	357
Crumb color								
3752	Purkof	70cy	68cy	65cy	70cy	60cy	73cy	63cy
3753	Redrock	78cy	80c	83c	78cy	83c	83c	80c
3754	Nittany	60cy	63cg	65cy	70cy	63cy	65cg	70cy
3755	Trumbull	65cy	70cy	68cy	68cy	68cy	72cy	70cy
3756	Am. Banner	70cy	65cy	55cy	60cy	60cy	63cy	65cy
Grain								
3752	Purkof	70	75	75	75	70	78	63
3753	Redrock	70	80	83	83	83	88	80
3754	Nittany	55	63	65	65	68	67	65
3755	Trumbull	58	68	75	75	80	75	72
3756	Am. Banner	60	58	50	55	58	55	53
Texture								
3752	Purkof	68	74	74	81	81	74	68
3753	Redrock	61	74	81	74	87	81	74
3754	Nittany	48	48	61	68	74	74	68
3755	Trumbull	48	55	74	74	81	78	70
3756	Am. Banner	48	48	48	48	55	48	48
Loaf type and crust color								
3752	Purkof	MH7	M6	M9	M9	M8	M8	MH7
3753	Redrock	MH7	M6	M10	M9	MH8	M8	MH8
3754	Nittany	MH6	MH5	M9	M8	M8	M9	MH8
3755	Trumbull	MH7	MH5	M10	M9	M9	M9	H8
3756	Am. Banner	M7	M5	M11	M8	H8	JH8	H8
Formula data								
Sugar (%)		2.5		5.0		5.0		5.0
Salt (%)		1.0		1.0		1.0		1.0
Yeast (%)		3.0		3.0		3.0		3.0
200°L. diastatic malt (g.)		—		—		0.15		0.3
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>		—		—		0.05		0.1
KBrO <sub>3</sub>		—		—		0.0005		0.001

TABLE 13.—Continued

NO.	VARIETY	FORMULA						
		1 <sup>1</sup>	1 <sup>2</sup>	2 <sup>1</sup>	2 <sup>2</sup>	3 <sup>1</sup>	3 <sup>2</sup>	4 <sup>1</sup>
Loaf volume (cc.)								
3752	Purkof	435	440	433	481	527	460	594
3753	Redrock	486	462	473	495	537	492	573
3754	Nittany	468	435	435	470	523	455	560
3755	Trumbull	484	450	465	490	523	470	643
3756	Am. Banner	405	385	396	426	465	428	502
Crumb color								
3752	Purkof	63cy	75cy	60cy	68cy	70cy	68cy	80c
3853	Redrock	84c	85c	85c	83c	80c	80c	90c
3754	Nittany	65cg	63cy	60cg	65cy	70cy	68cy	85c
3755	Trumbull	75cy	73cy	65cy	73cy	65cy	73cy	83c
3756	Am. Banner	60cy	60cy	60cy	63cy	60cy	68cy	75cy
Grain								
3752	Purkof	63	78	70	75	70	78	95
3753	Redrock	83	85	83	88	90	88	100
3754	Nittany	60	73	60	68	60	70	95
3755	Trumbull	75	78	65	80	70	80	90
3756	Am. Banner	53	58	50	58	50	60	78
Texture								
3752	Purkof	68	74	68	81	74	74	100
3753	Redrock	87	87	81	87	87	87	94
3754	Nittany	61	61	61	74	61	68	87
3755	Trumbull	74	68	68	74	74	74	87
3756	Am. Banner	48	48	48	61	48	61	74
Loaf type and crust color								
3752	Purkof	MH7	MH8	M7	M10	M11	M8	L11
3753	Redrock	M8	MH9	MH10	M10	M11	M8	L11
3754	Nittany	M8	H9	M8	M10	M11	M10	M11
3755	Trumbull	M9	MH9	M8	M10	M11	M10	L12
3756	Am. Banner	H8	H8	H8	M10	M11	M9	M11
Formula data								
Sugar (%)	5.0	5.0	5.0	5.0	6.0	5.0	5.0	
Salt (%)	1.0	1.0	1.0	1.5	1.0	1.5	1.5	
Yeast (%)	3.0	3.0	3.0	3.0	3.0	2.0	2.0	
200°L. diastatic malt (g.)	—	—	—	—	—	—	—	
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> (g.)	—	—	—	—	—	—	—	
KBrO <sub>3</sub> (g.)	0.0005	0.001	0.0005	—	—	—	—	0.001
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> (g.)	—	—	0.25	—	—	—	—	
Dry skim milk (%)	—	—	—	—	—	—	—	4.0
Shortening (%)	—	—	—	—	—	—	—	3.0

<sup>1</sup> Hand punched and molded.<sup>2</sup> Machine punched and molded.<sup>3</sup> Single bake.

rolls, when used for punching and molding, do not materially reduce the variability in the baking test. Possibly, if some changes were made on the sheeter, it could be recommended as a standard piece of equipment in the test baking of soft wheat flours.

*Comparison of baking formulas.*—As in the preceding year, a number of baking formulas were compared with the A.O.A.C. basic formula. In the majority of cases these tests were made with the machine method of punching and molding. The two most promising baking methods were repeated, hand punching and molding being used. The results are given in Table 13. Judged from previous experience, these five soft wheat varieties should rank, in order of decreasing strength, as follows: Purkof, Redrock, Trumbull, Nittany, and American Banner. The varieties actually rank themselves in the following order as far as decreasing quantities of flour protein are concerned: Trumbull, Redrock, Nittany, Purkof, American Banner. The Trumbull and Purkof samples are abnormal in respect to protein content, the Purkof being too low and the Trumbull too high for this particular series of samples.

The baking results are disappointing owing to the lack of differentiation between varieties. When loaf volumes are considered, the A.O.A.C. basic method places the weakest variety, American Banner, next in strength to Redrock. The stronger varieties, Trumbull and Nittany, are shown weaker than American Banner. This is the only method of all those tested that does not place American Banner as the weakest of the five varieties. It would appear, therefore, that the A.O.A.C. basic baking method is not suitable for evaluating soft wheat varieties.

Formulas B and C are considered most promising in the evaluation of soft wheat varieties. While the data given in Table 13 are not conclusive, these formulas have proved the most satisfactory over a period of years.

#### 1939 CROP SAMPLE EXPERIMENTS

Owing to a change of position this year the Associate Referee could not carry on as extensive studies as he had in the preceding two years. However, a series of different formulas was tested out with five standard soft wheat variety flours. The data resulting are presented in Table 14. A number of observations on these data may be interesting.

If the protein contents are of comparable amounts previous experience with these varieties would place them as follows in descending order of strength: Purkof, Redrock, Trumbull, Nittany, and American Banner. A prime prerequisite in any satisfactory baking method should be its ability to rank the varieties in the correct order. A second feature of importance would be that the baking procedure produces a good range in values between the stronger and weaker flours.

In these 1939 samples the A.O.A.C. basic formula ranks the flours from the different varieties of flour better than in previous years. It still gives

TABLE 14.—*Study of effect of baking formulas on 1939 soft wheat flours*

VARIETY	FORMULA AND METHOD											
	A.O.A.C.	A	B	C	D	E	F <sup>1</sup>	G <sup>1</sup>	H	I	J	K
Loaf volume (cc.)												
Purkof	572	611	689	681	735	786	631	667	577	729	759	802
Redrock	574	615	614	657	718	770	654	658	556	749	726	789
Nittany	523	563	591	642	722	722	571	646	568	717	751	736
Trumbull	544	586	600	664	690	734	599	661	557	734	758	775
Am. Banner	488	530	557	565	624	581	556	592	529	633	630	666
Crumb color												
Purkof	71ey	69ey	73ey	80e	83e	83e	70cy	71ey	70cy	76cy	78ey	81e
Redrock	79ey	76cy	79ey	84e	88e	89e	78cy	80e	75cy	81e	85e	85e
Nittany	68eg	64eg	68cy	76cy	81e	81e	69cy	66eg	68cy	78cy	81e	75cy
Trumbull	66cy	66ey	64cy	75cy	75cy	74cy	63cy	69cy	68cy	75cy	73cy	75cy
Am. Banner	60cy	59ey	63cy	65cy	71cy	65cy	58cy	60cy	65cy	73cy	70cy	73cy
Grain												
Purkof	75	74	81	85	90	91	74	78	73	91	89	89
Redrock	75	78	79	89	90	90	79	83	75	88	88	91
Nittany	61	61	66	79	84	83	64	61	66	84	85	85
Trumbull	66	70	74	80	83	84	63	73	73	85	85	86
Am. Banner	56	59	64	70	74	69	53	58	65	78	78	80
Texture												
Purkof	77	77	87	90	94	100	81	87	74	87	87	100
Redrock	81	81	84	90	90	100	87	87	74	87	87	100
Nittany	61	64	74	87	87	90	74	74	68	87	87	100
Trumbull	71	74	81	87	87	87	74	81	74	87	84	100
Am. Banner	48	61	61	74	74	74	61	61	64	77	74	87
Loaf type and crust color												
Purkof	L8	L10	F10	L10	F11	F11	L11	L11	M11	F12	F12	F12
Redrock	L8	L10	L9	L10	F10	L11	L11	L10	M11	F12	F12	F12
Nittany	M8	M10	L11	L10	L11	L11	M11	L11	M11	F12	F12	F12
Trumbull	M8	M10	L10	L10	L11	F11	L11	L11	M11	F12	F12	F12
Am. Banner	M8	M10	M11	M11	M11	M11	M11	M10	M11	L12	M12	L12
Formula data												
Sugar (%)	2.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	6.0
Salt (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.5	1.5	1.5	1.5
Yeast (%)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	2.0	2.0
Dry Skim Milk (%)	—	—	—	—	—	4.0	—	—	—	4.0	4.0	4.0
Shortening (%)	—	—	—	3.0	3.0	3.0	—	—	—	3.0	3.0	3.0
120°L. Diastalt (gm.)	—	—	—	—	—	—	—	—	—	—	—	0.25
200°L. Diastalt Malt (gm.)	—	—	0.15	—	0.15	0.15	—	0.15	—	—	—	—
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> (gm.)	—	—	0.05	—	0.05	0.05	—	0.05	—	—	—	—
KBrO <sub>3</sub> (mg.)	—	—	0.5	—	0.5	0.5	—	0.5	—	—	1.0	3.0

Procedure applying to above bakes unless otherwise stated:

1. 100 grams of flour (15% moisture basis).
2. Mixing 1.5 minute in Hobart-Swanson mixer.
3. Absorption variable.
4. 3-hour fermentation at 30°C.
5. Proof 55 minutes at 30°C.
6. Doughs hand punched and molded.
7. All baking values are the average of 4 bakes, duplicate bakes being made on 2 separate days.

<sup>1</sup> Instead of regular 3 hour fermentation, ferment 105 minutes. First punch after 70 minutes. Pan after additional 35 minutes. Proof 55 minutes.



a relatively low spread in results, however. Formulas B, E, and K gave the largest range in loaf volume values. Formulas E and K, which specify shortening and milk, are patterned after rich commercial-type formulas.

As the fundamental purpose of a baking test for soft wheat flours is that of testing flours it would appear that preference should be given to the simpler formulas that require fewer ingredients, each of which is a possible variable when used in the baking test. The use of shortening, together with dry milk solids, unquestionably improves the baked loaf, particularly internally, as well as from the standpoint of nutrition and palatability. These latter two points are considered of secondary importance insofar as soft wheat flours are concerned, because the baking test should prove most useful for classifying these flours and not testing them for bread-making potentialities.

When all points are considered it would appear that Formula B shows considerable promise when used for testing unbleached, experimentally milled flours. Its probable usefulness with bleached flours will largely depend upon the amounts and types of bleach used.

#### SUMMARY AND CONCLUSIONS

Study over a three-year period shows that the basic formula of the Association of Official Agricultural Chemists does not give the desired differentiation between soft wheat flours, and that 2.5 per cent of sugar is insufficient. At least 5 per cent sugar should be used.

Experiments with varying amounts of yeast and salt indicate that the recommended quantities of these ingredients may be used. The use of 1 per cent of salt and 3 per cent of yeast also proved satisfactory.

The use of 0.15 gram of high diastatic (200°L) malt, 0.05 gram of ammonium phosphate, and 0.5 mg. of potassium bromate per 100 grams of flour has increased the differentiation between samples. This is a modified malt-phosphate-bromate formula patterned after methods used in testing spring wheat bread flours.

Sufficient data are not available at this time to state definitely whether the use of shortening and milk is justified.

Experiments with sheeter rolls indicate that their purchase is not justified, at least so long as a skilled baker is making the tests. Where sheeter rolls are available it would appear that they may be used if desired.

A satisfactory dough-handling technic has been evolved; it employs a piece of canvas belting, a rolling pin, and a trackway with a clearance of 5/16 inch.

Mixing experiments show that the Hobart-Swanson mixer is satisfactory. The Hobart mixer equipped with a cake paddle also proved satisfactory.

## RECOMMENDATIONS\*

It is recommended—

(1) That the amount of sugar specified in the formula of the Association of Official Agricultural Chemists be increased to 5 per cent, and that with this exception the basic formula and technic be left unchanged.

(2) That a collaborative committee be appointed to test this revised basic formula against the modified malt-phosphate-bromate formula mentioned above, and that added studies include a formula with both milk and shortening superimposed upon the malt-phosphate-bromate formula.

(3) That this collaborative committee attempt to standardize a hand-punching and molding technic, which is an adaptation of the method originally suggested by Merritt, Blish, and Sandstedt, *loc. cit.* For punching, place the dough bowl-side down on a piece of canvas belting and roll each way from the center by means of a rolling pin operating on a wooden track that gives a clearance of 5/16 inch. The dough is inverted, the opposite ends overlapped and then placed, seam down, in the fermentation bowl. In molding, the punching operation just described is followed. However, after the opposite ends have been overlapped, the dough is inverted and turned parallel with the wooden strips. It is rolled each way from the center. The dough is then again turned over, and starting at the more remote end, rolled up by hand toward the operator. The seam is sealed and the dough is rolled lightly under the palm of the hand before being placed seam down in the pan.

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REPORT ON FLOUR-BLEACHING CHEMICALS

By DOROTHY B. SCOTT (U. S. Food and Drug Administration,  
New York, N. Y.), *Associate Referee*

Collaborative samples of flour were sent to six analysts for the determination of chlorine in the fat of flour by the method and instructions given in last year's report and in a paper, *This Journal*, 23, 498, 675 (1940).

The only essential changes were the increase of the quantity of fusion mixture from 15 to 20 grams and the increase in the ashing temperature of the muffle from 525° to 600°C. Instructions for obtaining a white ash by wetting and re-ashing were also given.

Most of the analysts' results were in close agreement, but some of those of two analysts were unsatisfactory. It is believed that increasing the ashing temperature to 600°C. may cause the porcelain to be attacked by the alkali in some instances.

One analyst, who obtained satisfactory results last year using 525°C. for ashing, reported two high results this year by using 600°C. J. L. Loughrey obtained very high blanks at a temperature of 600°C. but a

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 65 (1941).

blank of 0.40 mg. of chlorine at 525°C. This blank is in accord with the blanks obtained by other analysts.

After all the collaborative results had been received the Associate Referee analyzed the samples submitted and ran a series of blanks at 600°C. using nickel crucibles. The results of the blanks were consistently the same.

Nickel or iron crucibles for alkali fusions are advocated by Hillebrand and Lundell in "Applied Inorganic Analysis," page 20, and they are also specified in the method for the determination of iodine in thyroid in U. S. Pharmacopoeia XI, page 388.

The Associate Referee is convinced that nickel crucibles should be substituted for porcelain crucibles in the method if a temperature of 600°C. is used. Samples will be submitted again for collaboration after further experimentation with this change.

The results of the collaborators are shown in the table.

*Collaborative results (mg. chlorine per 1 gram of fat)*

COLLABORATOR	SAMPLE A 2.5 G. AGENE PER BBL. OF FLOUR	SAMPLE B UNBLEACHED	SAMPLE C 5 G. AGENE PER BBL. OF FLOUR	SAMPLE D BETA-CHLORA BLEACH
R. A. Barackman Victor Chem. Works Chicago	0.43*	0.63*	0.59	23.07
Anthony Hunt Food & Drug Adm. New York	0.29 } Av. 0.30 } 0.30	0.10 } Av. 0.13 } 0.12	0.43 } Av. 0.47 } 0.45	18.31 } Av. 16.86 } 17.59
George Kepple Food & Drug Adm. Minneapolis	0.23 } Av. 0.22 } 0.23	0.08 } Av. 0.09 } 0.09	0.40 } 0.49 } Av. 0.40 } 0.43	17.25 } Av. 16.94 } 17.10
Hugh K. Parker Wallace, Tiernan Co. Newark	0.37	0.12	0.57	21.65
J. L. Loughrey Food & Drug Adm. Boston	2.99*	2.49*	1.88*	24.5*
Manuel Tubis Food & Drug Adm. Philadelphia	0.17 } Av. 0.23 } 0.20	0.11 } Av. 0.09 } .010	0.44 } Av. 0.46 } 0.45	18.77 } Av. 18.14 } 18.46
<i>Nickel Crucibles</i>				
D. B. Scott	0.27	0.10	0.44	18.34
Average	0.26	0.10	0.47	18.81

\* Results not counted in average.

## COMMENTS

*R. A. Barackman.*—Our first test on Sample B resulted in a dark ash, which could not be burned white. We repeated this test but used 200 grams of flour in place of 500 grams and therefore obtained a white ash. The ash of Sample D became fused. Work on this sample was repeated to obtain a white fluffy ash.

*George Kepple.*—Difficulty was experienced in obtaining a carbon-free ash except for Sample D, where the amount of fat is less. However, a second heating after moistening with water usually eliminated the carbon.

*J. L. Loughrey.*—Because of the trouble with the blanks and because it was impossible to run duplicate analyses on the flour, I do not feel too confident of the results reported.

*Hugh K. Parker.*—I had no difficulty with the method, but I am not too sure of the results since I worked under rather adverse conditions, due to the fact that occasionally during the analysis there were fumes of a volatile chlorine compound in the room as a possible contaminant. For this reason my results may be higher than those of the other collaborators.

*Manual Tubis.*—If desired, the quantity of fat can be increased by filtering the petroleum benzine extract the second time through only a part of the flour cake with the minimum suction necessary.

## RECOMMENDATIONS\*

It is recommended—

- (1) That the study of the method for the determination of chlorine in the fat of flour be continued.
- (2) That the method for the determination of benzoyl peroxide in flour be further studied.
- (3) That the Munsey method for the determination of carotenoid pigments in flour be further studied.

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No report on CO<sub>2</sub> in self-rising flour was given by the associate referee.

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## REPORT ON MILK SOLIDS IN MILK BREAD BUTTERFAT IN MILK BREAD

By V. E. MUNSEY (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The previous collaborative studies on this subject indicated the need for improvement in the methods for the determination of milk solids in bread. As a result of these studies, several collaborators have mentioned difficulties in obtaining the complete saponification of the fat extracted from bread. Further experimentation showed that the method of saponification could be changed to effect complete saponification. This change from saponification with straight 50 per cent sodium hydroxide to a saponification with the official glycerol-soda mixture, as given under the chapter on Oils, Fats, and Waxes, *Methods of Analysis*, A.O.A.C., 1940,

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 65 (1941).

p. 229, was the essential difference in the method as studied this year.

Two samples of milk bread, identified as 145 and 146 and one sample with one-half the milk solids of milk bread, identified as 148, were submitted to nine collaborators that had indicated their willingness to co-operate in this work. The bread identified as 145 was prepared from a known amount of 81 per cent butterfat butter and dry skim milk so that

TABLE 1.—*Fat, fat number, and per cent butterfat in bread samples*

ANALYST	H <sub>2</sub> O		FAT—MPB		BLANK	FAT NUMBER			BUTTERFAT (%)		
(Sample 145)											
1	8.95 8.92	8.94	6.84 6.84	6.84	0.7	15.10 —	14.50 14.50	14.70	2.86 —	2.71 2.71	2.76
2	9.01 8.95	8.98	7.04 7.03	7.04	0.5	12.60 12.00	12.00 12.70	12.30	2.30 2.15	2.15 2.33	2.23
3			6.02 6.01	6.02	1.45	15.40 15.90	16.40 15.40	15.80	2.58 2.69	2.80 2.58	2.67
4	8.96 8.96	8.96	6.98 6.98	6.98	1.0	12.00 11.80	10.80 10.80	11.40	2.13 2.08	1.83 1.83	1.98
5	8.75 8.87	8.81	7.04 6.92	6.98	1.2	12.60 13.70	12.80 13.80	13.20	2.28 2.56	2.33 2.59	2.44
(Sample 146)											
1	9.59 9.58	9.59	5.87 5.95	5.91	0.7	18.70 19.10	18.70 16.80	18.30	3.14 3.22	3.14 2.75	3.06
2	9.68 9.68	9.68	6.03 6.07	6.05	0.5	15.60 14.90	14.50 14.60	14.90	2.56 2.41	2.32 2.34	2.41
3	9.12 9.16	9.14	5.50 5.69	5.60	1.45	18.10 18.00	18.30 17.70	18.00	2.86 2.84	2.90 2.78	2.84
4	9.59 9.52	9.56	5.97 5.93	5.95	1.0	13.00 11.70	12.00 12.20	12.20	1.97 1.70	1.76 1.80	1.80
5	9.37 9.29	9.33	5.92 6.06	6.00	1.2	15.00 15.90	15.30 15.40	15.40	2.41 2.60	2.47 2.49	2.49
(Sample 148)											
1	8.54 8.53	8.54	5.56 5.46	5.51	0.7	11.50 11.90	11.70 11.70	11.70	1.58 1.66	1.62 1.62	1.62
2	8.49 8.45	8.47	5.89 5.89	5.89	0.5	8.30 8.60	8.30 9.00	8.60	1.01 1.07	1.01 1.16	1.07
3	8.55 8.53	8.54	5.29 5.54	5.42	1.45	12.40 11.90	10.10 10.10	11.10	1.74 1.64	1.28 1.28	1.48
4	8.63 8.51	8.67	5.90 5.84	5.87	1.0	5.30 5.50	4.90 5.40	5.30	0.36 0.41	0.28 0.38	0.36
5	7.98 8.04	8.01	5.80 5.62	5.71	1.2	8.00 10.00	8.30 10.30	9.20	0.91 1.33	0.98 1.39	1.16

the bread contained 2.49 per cent added butterfat. The bread identified as 146 was made with 4 per cent butterfat liquid milk so that the bread contained 2.65 per cent added butterfat. The bread identified as 148 was made from equal parts of water bread (no butterfat added) and Sample 145 so that the added butterfat content was 1.25 per cent. The fat number on the fat extracted from the water bread was 3.6, from the fat used in sample 145 it was 31.1, and from the milk used in sample 146 it was 32.0, respectively. These values were used in the calculation of the amount of butterfat in these breads. The results from five collaborators are given in Table 1.

It will be observed that the amount of total fat is in good agreement, with the exception of that of Analyst 3, which runs consistently low on all samples. The results on the amount of added butterfat show more variation than on the total fat, which would indicate the variation in results associated with the fat number. Even though the results of different collaborators on the amount of added butterfat show considerable variation, the results, except for Analyst 4, are a reasonably close estimation of the amount of butterfat added.

Sincere appreciation is extended to the following collaborators:

Iman Schurman, Cincinnati, Ohio;  
W. C. Woodfin, Atlanta, Ga ;  
L. W. Ferris, Buffalo, N. Y. ;  
R. H. Johnson, Seattle, Wash.,

all of the Food and Drug Administration, Federal Security Agency.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That further study be made of the method discussed.
- (2) That the lactose procedure with any necessary modifications, *Cereal Chem.*, 13, 541 (1936), for the determination of milk solids in bread be more extensively studied collaboratively.

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No report on cold water extract flour was given by the associate referee.

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### REPORT ON PROTEOLYTIC ENZYMES

By QUICK LANDIS (The Fleischmann Laboratories,  
New York, N. Y.), *Associate Referee*

A simplification of the gelation rate method<sup>1</sup> has finally been devised (reported at the A.C.S. Meeting, September 1940). In order to determine the general reaction to such a method, an invitation for collaboration was

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 65 (1941).

<sup>1</sup> *Cereal Chem.*, 15, 91-101 (1938).

transmitted and two collaborators generously signified their willingness to cooperate.

The collaborators were provided with the necessary special apparatus and a sample of the substrate used by the Associate Referee. The generalized procedure for calibrating technic was thus not required, and the directions could be made quite specific. The results are given in Table 1.

TABLE 1.—*Collaborative results*

FLOUR	A	COLLABORATOR		A-C	MEAN	AVERAGE DEVIATION  per cent
		B	C			
A-Clear	.60 .72 > .66	.43 .49 > .46	.50 .51 > .51	.15	.54	11
B-Patent	.52 .55 > .54	.37 .42 > .40	.32 .35 > .34	.20	.42	16
C-Same	.50 .57 > .54	.21 .16 > .19	.34 .32 > .33	.21	.35	30
D-Cake	.29 .26 > .27	.16 .13 > .15	.15 .14 > .15	.12	.19	31

The following comments were received:

*Collaborator A.*—It is the only satisfactory method that I have yet seen for determining proteolytic activity in materials as low in such activity as our patent flour. On the other hand, . . . it would be difficult to use this technic as a routine determination.

*Collaborator B.*—1. The method is rather long and a little too sensitive for ordinary work. 2. Certain time intervals may have to be selected for the readings since during certain combinations of elapsed time and apparent viscosity, the sensitivity of the test is very much greater than during others. 3. The test is an improvement over the former method in the temperature range especially.

It is interesting to note that the results of Collaborator A were consistently about 0.2 milli-unit higher than those of Collaborator C, while those of Collaborator B were somewhat irregular. The comments of Collaborator B are well made, and further experiments should be undertaken to define the optimum range.

Theoretically no range should be permitted; i.e. all readings should be made at constant apparent viscosity. However, this is not feasible, and so a moderately complicated series of mathematical calculations is provided to widen the range.

Although these results indicate mean deviations as great as 30 per cent may be expected between laboratories, the Associate Referee believes that the method is worthy of further consideration in the light of the comments of the collaborators. It is therefore recommended\* that further investiga-

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 65 (1941).

tion of this method be prosecuted, with particular attention given to the optimum range and to further simplification.

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No report on color of flour and bread was given by the associate referee.

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No report on soya flour in foods was given by the associate referee.

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## REPORT ON WHOLE WHEAT FLOUR

By C. S. LADD (North Dakota State Laboratories Department,  
Bismarck, N. D.), *Associate Referee*

The investigation of the applicability of the cellulose determination of Kurschner-Hanak<sup>1</sup> to whole wheat products was continued by the Associate Referee.

It seemed advisable to investigate further the merits of the variations of the Kurschner-Hanak reagent, *This Journal*, 23, 508 (1940), and overcome, if possible, the difficulty of filtration. Owing to the difficulties presented in standardizing a percolation unit, direct digestion and Gooch filters were used.

Modified Gooches were prepared by placing 3–4 grams of acid-digested, ignited sand on the mat. The Gooches were washed, dried at 105°–110°C., cooled to room temperature in a desiccator, and weighed with a Gooch counter-poise that had been treated in the same manner to eliminate the errors due to variation in the weight of the Gooches.

One gram samples of dry, defatted material were weighed into 400 ml. beakers, digested 30 minutes with 60 ml. of the reagent under a round-bottomed condenser, filtered through the prepared Gooch, washed well, and dried to constant weight. Gooches were ashed at 600°C. and used as long as the mat remained in good condition.

Determinations were made on various materials to compare the time of filtration of the three reagents. Reagent 1 (60 ml. 80% HAc and 1.5 ml. HNO<sub>3</sub>) filtered slowest. Reagent 2 (Reagent 1 plus 2 grams of trichloroacetic acid) filtered more rapidly, while Reagent 3 (Reagent 1 plus 1.5 ml. of concentrated HCl) filtered the fastest.

As it had been stated in the previous report, *Ibid.*, that filter paper was degraded by the reagents, determinations were made to compare the recovery of cellulose from filter paper.

Reagent 1	Reagent 2	Reagent 3
95.6 %	93.9 %	92.1 %

Results of determinations made on a graham flour with the different reagents are given on a dry, fat-free basis.

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<sup>1</sup> *Untersuch. Lebensm.*, 59, 484–494 (1930).



Reagent 1	Reagent 2	Reagent 3
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
3.76	3.88	3.44
3.98	3.96	3.40

Reagent 3 gave white cellulose, while Reagents 1 and 2 gave light brown cellulose.

Further determinations on the graham flour were made with varying normality of the solvent. Glacial acetic acid plus 1.5 ml. of nitric acid plus 1.5 ml. of hydrochloric acid for each 60 ml. was found to be about 17 normal. This was diluted with water to lesser normalities, and the following results were obtained.

Normality	17	16	15	14	13	12	11	10	8
Cellulose (%)	3.75	3.41	3.24	3.25	3.43	3.53	3.78	3.85	4.31

Swedish filter paper was used for a similar determination and recovered cellulose recorded.

Normality	17	16	15	14	12	10
Cellulose (%)	94.85	93.32	92.09	92.16	94.62	97.76

Difficulty due to extreme super-heating during digestion was encountered until the size of sample was cut down to 70-90 mg. Thus the absolute value of these figures may be questioned because of the small size of sample, but their relative values show agreement with the graham flour determinations. The reagents of high normality (17 and 16) were not considered suitable since they were either slow or failed to break up the particles of filter paper completely. Use of reagents below 12 normal resulted in a light brown cellulose on the graham flour. Thus normalities of 14 or 15 were considered most effective. The variations on the graham flour determinations with reagents of these normalities were within experimental tolerance, and it was therefore decided that Reagent 3, which was found to be 14 normal, did not require change of composition.

To determine the reliability of the cellulose determination on a practical basis, analyses were made of authentic bread samples and their ingredients. Several determinations had to be abandoned after it was discovered that the use of reagent a day or two old gave slightly higher results. Freshly prepared Reagent 3 was used for the following determinations, and the individual results are reported on a dry, fat-free basis.

	WHOLE WHEAT FLOUR	WHITE FLOUR	100% W. W. BREAD	50% W. W. BREAD	100% W. W. DOUGH	50% W. W. DOUGH
Cellulose (%)	2.74	0.28	2.76	1.66	2.65	1.62
	2.75	0.26	2.75	1.60	2.62	1.65
	2.88	0.30	2.84	1.75	2.62	1.74
	3.08	0.33	2.71	1.71	2.61	1.74
	3.03	0.34	2.57	1.68	2.67	1.65
	3.14	0.35	2.59	1.59	2.55	1.59
Average	2.94	0.31	2.70	1.67	2.62	1.67
Crude Fiber	2.89	0.86	3.32	2.46	3.09	2.06
A.O.A.C.	2.91	0.79	3.35	2.36	3.28	2.09

Using data from the bread formulas and the results from the analyses the Associate Referee calculated the cellulose content for the 100 per cent whole wheat bread and the 50 per cent whole wheat bread. The analysis of the 100 per cent whole wheat bread showed a cellulose content of 2.70 per cent against a calculated value of 2.78 per cent. The analysis of 50 per cent whole wheat bread showed a cellulose content of 1.67 per cent against a calculated value of 1.53 per cent.

Similar calculations were made from crude fiber determinations. The analysis of 100 per cent whole wheat bread showed a crude fiber content of 3.33 per cent against a calculated value of 2.75 per cent. The analysis of 50 per cent whole wheat bread showed a crude fiber content of 2.41 per cent against a calculated value of 1.76 per cent.

The flour content of the breads was calculated from the analyses with the following results: 100 per cent whole wheat bread calculated 91.84 per cent whole wheat flour against an actual value of 94.85 per cent whole wheat flour; 50 per cent whole wheat bread calculated 51.36 per cent whole wheat flour against an actual value of 47.20 per cent whole wheat flour. Thus flour content was determined within 4.2 per cent on a dry, fat-free basis and within 2 per cent on the original basis.

Individual results on the bread project did not vary more than 0.10 per cent from the average, with the exception of the whole wheat flour, which varied as high as 0.20 per cent. Part of this variation was believed due to the lack of uniformity of the sample, which contained some coarse bran particles. Perhaps some variation might be eliminated by an increase in size of the sample and volume of reagent.

The use of sand as a filtering aid increases the rate of filtration and permits more adequate washing of cellulose. Although it is believed material containing much higher cellulose content could be filtered by breaking up the cellulose film that forms on the sand during filtration insufficient work has been done with high cellulose content materials to confirm this belief.

Comparison of the different reagents showed Reagent 3 to be the most satisfactory on comparing time of filtration and the appearance of the final cellulose, but it also degraded the cellulose to a large extent.

Cellulose determinations by the use of Reagent 3 on the bread project gave results in closer agreement to theoretical values than those obtained by the present crude fiber method, and therefore merits further investigation.

It is recommended that further investigation be conducted and that the method be subjected to collaboration.

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No report on phosphated flour was given by the associate referee.

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## REPORT ON STEROLS

By E. O. HAENNI (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The development of a method for the determination of sterols in farinaceous products was undertaken for the purpose of using the sterol content of such products as an index of the egg content. The Associate Referee has been working on this problem for several years in conjunction with the development of a method for the estimation of the cholesterol in eggs. The results of the work have been presented in a contributed paper, *This Journal*, 24, 119 (1941). The method, as described in detail in that paper, provides for the estimation of the unsaponifiable matter and the sterols in alimentary pastes and in their farinaceous ingredients. The unsaponifiable matter determination does not materially increase the working time of the sterol method, and it serves to indicate when it is advisable to add cholesterol to the unsaponifiable matter before proceeding with the sterol determination. Furthermore, the content of unsaponifiable matter in con-

TABLE 1.—*Collaborative results for unsaponifiable matter and sterol in noodles (dry basis)*

COLLABORATOR	UNSAAPONIFIABLE MATTER	STEROL (AS CHOLESTEROL)
	<i>per cent</i>	<i>per cent</i>
S. Alfend, St. Louis	0.31	0.193
	0.31	0.196
F. J. McNall, Cincinnati	0.32	0.195
	0.33	0.184
G. Kirsten, New York	0.31	0.191
	0.31	0.190
J. A. Schuldiner, New York	0.31	0.181
	0.30	0.177
H. W. Gerritz, San Francisco	0.33	0.193
	0.34	0.192
E. O. Haenni	0.30	0.192
	0.32	0.192
	[0.31]	[0.192]*
Maximum	0.34	0.196
Minimum	0.30	0.177
Average	0.32	0.190
Av. dev.	0.01	0.004

\* Results in brackets omitted from averages.

junction with the sterol content of a noodle may serve to detect sophisticated products in some instances.

A sample of noodles containing 5.6 per cent of egg-yolk solids (dry basis) was sent to collaborators with copies of the directions as given under "Revised Method" in the Experimental Part of the paper referred to above. They were asked to determine the total solids, unsaponifiable matter, and sterol (as cholesterol) in the sample. The results calculated on the dry basis are given in Table 1.

The results are in general very satisfactory. The low results for sterol obtained by one collaborator may be due to the use of a bromine reagent over two weeks old. Unfortunately the Associate Referee did not specify that the bromine reagent used should be reasonably fresh. This may not account for the low results obtained, but the short time available before the meeting did not permit the Associate Referee to decide. The collaborators did not report any particular difficulty with the method, but two laboratories found the preparation of the sodium hypochlorite inconvenient, evidently because of the lack of readily available chlorine. One laboratory suggested the use of commercial hypochlorite solutions. This possibility had been given some consideration by the Associate Referee, but it was concluded to be preferable (if the sterol method was shown to be satisfactory) for each laboratory to acquire one of the small and inexpensive refillable lecture table cylinders of chlorine now available on the market. These contain 1 pound of the gas, which is sufficient for hundreds of determinations. The Associate Referee considers that Gerritz aptly summed up the methods in stating: "The methods are long and require numerous manipulations as might be expected for such substances, but appear to be capable of reproducible results when one becomes familiar with them." Since the collaborative work shows that the method also gives concordant results by analysts in different laboratories, the Associate Referee believes it should be adopted as official, first action.

The Associate Referee expresses sincere thanks to the collaborators for their cooperation.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method developed for the determination of unsaponifiable matter in noodles and farinaceous ingredients of noodles be adopted as official, first action.

(2) That the method developed for the determination of sterols in noodles and farinaceous ingredients of noodles be adopted as official, first action.

(3) That these methods be applied to other farinaceous egg-containing products and further studied collaboratively.

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No report on corn products was given by the associate referee.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 65 (1941).

No report on oat products was given by the associate referee.

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No report on rye and buckwheat was given by the associate referee.

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## REPORT ON BARLEY AND RICE PRODUCTS

By ALLAN D. DICKSON (Bureau of Plant Industry, U. S. Department of Agriculture, Madison, Wis.), *Associate Referee*

In 1939 the methods for moisture, protein, fat, crude fiber, and ash given for wheat flour were applied to barley, pearled barley, and malt in a preliminary study. From this study, on which no report was made, it appeared that the methods were satisfactory, with the possible exception of the 130° air oven method and the method for crude fat when applied to malt. This year a collaborative study of these methods was undertaken. The results are summarized in this report.

Two samples each of barley and malt and one sample each of pearled barley and rice were prepared for analysis and submitted to four other laboratories. The three collaborators reporting, whose willing cooperation made this report possible, were:

F. A. Collatz, General Mills, Minneapolis, Minn.

M. H. Neustadt, Agricultural Marketing Service, Washington, D. C.

Emily Grewe, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

The following determinations from *Methods of Analysis*, A.O.A.C. 1940, were studied. Duplicate tests were reported by each analyst.

*Moisture*.—A—Vacuum Oven method, Chapter XX, Sections 2–3. B—103–104° Air Oven Method, Chapter XIV, Sections 39–41.

*Protein*.—Chapter XX, Section 22, the factor 6.25 being used to convert nitrogen to protein.

*Crude Fat or Ether Extract*.—Chapter XX, Section 10, except that preliminary water extraction was used on the malts.

*Crude Fiber*.—Chapter XX, Section 12.

*Ash*.—Chapter XX, Section 5.

The detailed data from this collaborative study are presented in Table 1.

## COMMENTS ON METHODS

*F. A. Collatz*.—Reported discrepancy in fat values on rice when using samples from air oven and vacuum oven methods. Lower fat value and higher moisture value indicated some ether-soluble material removed in vacuum oven. This might warrant further study.

*M. H. Neustadt*.—Suggested substitution of petroleum benzin (Skellysolve F) for dry ether, to eliminate need for drying ether and drying sample preliminary to extraction.

*Collaborative results on moisture, protein, fat, crude fiber, and ash in barley and rice products*

COLLABORATOR	BARLEY NO. 1		BARLEY NO. 2		MALT NO. 1		MALT NO. 2		PEARLED BARLEY		RICE	
Moisture—Vacuum Oven Method (%)												
1	11.7	11.7	10.7	10.8	5.6	5.7	8.8	8.8	12.1	12.1	12.5	12.7
2	11.7	11.7	10.9	10.9	5.8	5.8	8.9	8.9	12.2	12.1	12.4	12.5
3	11.4	11.4	10.6	10.6	5.2	5.3	8.5	8.5	11.8	11.8	11.9	11.8
4	11.2	11.2	10.3	10.3	—	—	—	—	11.8	—	11.9	11.9
Av.	11.5		10.6		5.6		8.7		12.0		12.2	
Moisture—103–104° Air Oven method (%)												
1	11.3	11.4	10.5	10.5	5.8	5.7	8.7	8.6	11.7	11.9	11.7	11.8
2	10.9	10.9	9.9	9.9	4.6	4.8	8.0	8.1	11.3	11.3	11.2	11.3
3	10.8	10.8	10.1	10.1	4.7	4.8	9.0	8.0	11.4	11.4	11.5	11.5
4	—	—	—	—	4.6	4.5	7.9	7.8	—	—	—	—
Av.	11.0		10.2		4.9		8.1		11.5		11.5	
Protein (Nitrogen×6.25) (%)												
1	11.94	11.87	9.19	9.25	11.94	12.00	9.19	9.19	10.87	11.00	8.50	8.37
2	12.25	12.06	10.31	10.12	12.44	12.31	9.44	9.50	11.50	11.25	8.37	8.87
3	11.69	11.75	8.94	9.00	11.69	11.75	9.12	9.06	10.81	10.75	8.31	8.37
4	11.87	11.94	9.00	8.87	11.25	11.37	9.25	9.37	13.06	12.87	8.12	8.00
Av.	11.92		9.33		11.84		9.26		11.51		8.36	
Crude Fat or Ether Extract (%)												
											(1.77)	(1.73)
1	2.27	2.23	2.19	2.16	1.00	0.97	1.20	1.17	0.81	0.81	2.38	2.38
2	2.01	1.93	2.08	1.98	1.72	1.73	1.81	1.83	1.31	1.36	1.67	1.70
3	2.08	2.06	2.30	2.28	1.83	1.86	1.91	1.93	1.24	1.28	2.55	2.50
4	2.29	2.12	2.41	2.45	1.65	1.70	1.76	1.71	1.38	1.23	2.58	2.72
Av.	2.12		2.23		1.56		1.66		1.18		2.31	
Crude Fiber (%)												
1	6.17	6.12	5.72	5.75	6.43	6.48†	5.61	5.52	1.04	1.02	1.00	0.95
2	5.42	5.42	5.12	5.25	6.16	6.38	5.57	5.57	0.54	0.63	0.61	0.60
3	6.14	6.15	5.55	5.51	6.63	6.61	6.07	6.10	0.98	1.04	1.06	1.07
4	—	—	—	—	—	—	—	—	—	—	—	—
Av.	5.90		5.48		6.45		5.74		0.88		0.88	
Ash (%)												
1	2.45	2.43	3.07	3.05	2.29	2.29	2.63	2.62	1.11	1.11	1.11	1.10
2	2.53	2.43	3.12	3.13	2.32	2.35	2.65	2.62	1.13	1.12	1.08	1.00
3	2.36	2.37	2.96	2.96	2.26	2.26	2.58	2.58	1.11	1.10	1.10	1.09
4	2.47	2.47	3.04	3.06	2.29	2.26	2.63	2.76	1.15	1.15	1.11	1.11
Av.	2.44		3.05		2.29		2.63		1.12		1.09	

\* Lower values obtained on sample dried in vacuum oven.

† Reported values divided by 2. Obviously an error in calculation.

## CONCLUSIONS AND RECOMMENDATIONS

Except for the ash values, the results show more variation than desirable, particularly those for fat and crude fiber. It is believed that the agreement with these two latter methods may be appreciably influenced by the type of equipment used, and that closer agreement could be obtained with standardized equipment.

Since these methods are rather widely used for barley and rice products, and the results of this study indicate that they are generally applicable, it is recommended\* that they be adopted as tentative for such products. Also that the factor 6.25 be used in converting nitrogen to protein.

## REPORT ON BAKED PRODUCTS OTHER THAN BREAD

By STEPHEN S. VORIS (Loose-Wiles Biscuit Co.,  
New York, N. Y.), *Associate Referee*

Last year's report covered a collaborative study of the analysis of three types of biscuit products for moisture, ash, protein, and fat by the acid hydrolysis method.

This study has since been extended to the three following additional types of products: 1. Whole Wheat Cereal, 2. Graham Crackers, and 3. Cake.

These samples were ground and forwarded to the collaborators. The sample of cake was air dried before being ground. The collaborators were requested to use the following methods of analyses on these samples, as given in *Methods of Analysis, A.O.A.C.*, 1935:

Moisture	—Air oven method, p. 207, 4.
Ash	—Method 1, p. 224, 56.
Protein	—p. 224, 58.
Crude fiber	—p. 224, 60.
Fat	—Acid hydrolysis method, p. 224, 59.

Since the determination of crude fiber involves the ether extraction of a weighed sample, the collaborators were requested to use this ether extract in the determination of fat by the Bailey-Walker method for comparison with the fat obtained by the acid hydrolysis method.

The results of these various analysis are shown in Table 1. The results obtained by the various collaborators for moisture, ash, and protein are very consistent, and in conjunction with the results of these determinations obtained on last year's samples, *This Journal*, 23, 537 (1940), indicate that these methods are satisfactory.

These methods have been recommended as tentative, *Ibid.*, 74, and the Associate Referee believes that on the basis of the results obtained by the collaborators they should be made official, first action.

The results obtained on crude fiber and fat were not so encouraging. The method for crude fiber has been recommended as official, first action,

\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 65 (1941).

*Ibid.*, 17, 59 (1934), but final action should not be taken until more consistent results can be obtained. It is apparent that the method for crude fiber requires further study. A comparison of the results of the fat determination by the Bailey-Walker method and by the acid hydrolysis method show that the Bailey-Walker method gives results consistently about 1.5 per cent lower than does the acid hydrolysis method. Apparently ether extraction does not remove all the fat. Because of the quantity of fat not removed by the ether extraction, it is believed that this fat is held by a surface absorption rather than by mechanical occlusion. T. C. Taylor and J. M. Nelson<sup>1</sup> state that a small amount of the fat in maize starch seems to exist in combination with the starch and is not removed by solvents, but is set free by hydrolysis. Since the quantity of fat not removed by ether extraction is relatively constant this introduces a greater variation between the Bailey-Walker method and the acid hydrolysis method on low fat samples than on high fat samples. It is believed that the acid hydrolysis method is more suitable, since it appears to give a more nearly correct indication of the fat content.

Table 2 gives the comparison of the fat determination by the acid hydrolysis method on last year's samples and this year's samples by six collaborators. In every case at least one and usually two of the collaborators obtained results that vary sufficiently from the mean of those of the other collaborators to invalidate the determination. These variations are fairly well scattered among the collaborators, with the exception of Collaborator 4, who shows consistently low results. In the case of three of the samples, Collaborator 4 obtained results so much lower than the mean of the others that they could not be included in the averages, and in the case of the other three samples the results obtained by Collaborator 4 were the minimum that were acceptable. The other collaborators who submitted results varying appreciably from the mean of the others generally obtained results that were too high, which might be accounted for by the carry over of material not fat in the ether extraction after hydrolysis.

The tendency for low results obtained by Collaborator 4 may possibly be explained on the basis of some investigations carried on in the Associate Referee's laboratory. In the course of the analysis of the sample of graham crackers for fat by the acid hydrolysis method the sample undergoing hydrolysis was overlooked and allowed to stand for 12 hours before extraction. The method calls for a hydrolysis period of 30-40 minutes. The fat content obtained on the extraction of this sample was unusually low, and further experiments were made to determine the effect of the length of the hydrolysis period on the fat content. The following results were obtained:

Hydrolysis Time hours	Fat Content per cent
$\frac{1}{2}$	10.45
2	10.27
12	8.64

<sup>1</sup> *J. Am. Chem. Soc.*, 42, 1726 (1920).



TABLE 1.—Collaborative results (%)

COLLABORATOR	SAMPLE 1.—WHOLE WHEAT CEREAL						SAMPLE 2.—ORANAM CRACKER						SAMPLE 3.—CAKE					
	MOISTURE	ASH	PRO-TEIN N X 5.7	CRUDE FIBER	BAILEY-WALKER	FAT ACID HYDROLYSIS	MOISTURE	ASH	PRO-TEIN N X 5.7	CRUDE FIBER	BAILEY-WALKER	FAT ACID HYDROLYSIS	MOISTURE	ASH	PRO-TEIN N X 5.7	CRUDE FIBER	BAILEY-WALKER	FAT ACID HYDROLYSIS
1	8.05	2.27	10.21	2.65	1.48*	2.72	6.20	2.98	8.10	0.79	8.89	10.08	5.55	0.58	7.77	0.47*	5.39*	6.02
2	9.0	2.35	10.20	2.24	0.92	—	7.2	3.04	8.60	0.78	8.71	—	5.8	0.61	8.20	0.07*	4.87	—
3	9.44	2.48	10.28	1.90	1.01	2.66	7.36	3.16	8.84	0.95	8.40	10.73*	6.25	0.60	8.40	0.24	4.69	6.85
4	9.11	2.16	10.67	1.82	—	2.76	7.62	3.14	8.90	1.05	—	10.06	6.17	0.62	8.40	0.32	—	8.38*
5	8.7	2.35	10.1	1.06*	0.53*	1.38*	7.2	3.14	8.4	1.33	6.25*	10.01	6.0	0.62	8.1	0.37	6.46*	5.40
6	9.03	2.27	10.03	1.95	1.04	2.65	7.23	3.05	8.53	1.23	8.44	11.47*	6.05	0.62	8.03	0.35	4.69	10.35*
7	8.97	2.35	10.56	2.10	1.13	1.88*	6.81	3.02	8.73	1.02	8.56	10.03	6.31	0.60	8.24	0.33	4.59	6.46
8*	—	—	—	—	—	—	3.86	3.27	8.67	—	8.70	9.45	—	—	—	—	—	—
Max.	9.44	2.48	10.67	2.65	1.13	2.76	7.62	3.16	8.90	1.33	8.89	10.08	6.31	0.62	8.40	0.37	4.87	6.85
Min.	8.05	2.16	10.03	1.82	0.92	2.65	6.20	2.98	8.10	0.78	8.40	10.01	5.55	0.58	7.77	0.24	4.59	5.40
Av.	8.90	2.32	10.29	2.11	1.03	2.70	7.09	3.08	8.59	1.02	8.60	10.05	6.02	0.61	8.16	0.32	4.71	6.18

\* Not included in average.

It will be seen that increasing the period of hydrolysis above that given in the method will give low results. Charring was observed in the samples hydrolyzed for more than 1 hour, and it seemed to increase progressively as the hydrolysis period was lengthened. This charring increases the viscosity of the mixture and makes it difficult to extract the fat. It is surprising to note that although only three points were determined, they appear to fall on a straight line.

It is believed that the inconsistencies obtained for crude fiber and fat by the acid hydrolysis method are more the result of difficulties in technic than in the methods themselves; however, it is believed that these methods require further study.

The Associate Referee recommends that the following methods for the analyses of baked products other than bread not containing fruits, *Methods of Analysis, A.O.A.C.*, 1940, be made official, first action:

Total Solids	p. 229, 64.
Ash	p. 230, 67.
Protein	p. 231, 69.

The determination of crude fiber and of fat by the acid hydrolysis method requires further study and it is recommended that the study of these methods be continued. It is further recommended that collaborative studies be made of the determination of moisture in baked products other than bread containing fruits.

The Association appreciates the cooperation of the following collaborators in this work:

- W. F. Allen, Kellogg Company, Battle Creek, Mich.  
 B. L. Kaspin, Bureau of Agr. Chem. and Eng., Washington, D. C.  
 F. A. Collatz, General Mills Inc., Minneapolis, Minn.  
 A. A. Hochman, Quartermaster Supply, Chem. Lab., Brooklyn, N. Y.  
 S. Laufer, Schwarz Laboratories, New York, N. Y.  
 L. R. Olsen, International Milling Co., Minneapolis, Minn.  
 O. I. Struve, Eastern States Cooperative Milling Corp., Buffalo, N. Y.

TABLE 2.—*Fat-acid hydrolysis method (%)*

COLLABORATOR	A VANILLA WAFFER	B SHORTBREAD	C SODA CRACKER	1 WHOLE WHEAT CEREAL	2 GRAHAM CRACKER	3 CAKE
1	19.22	24.33	12.79	2.72	10.08	6.02
2	—	—	—	2.66	10.73*	6.85
3	20.91*	24.32	12.79	2.76	10.06	8.38*
4	18.34	23.26*	10.91*	1.38*	10.01	5.40
5	19.15	24.65	23.00*	2.65	11.47	10.35*
6	18.72	24.17	12.88	1.88*	10.03	6.46
7	19.57	25.26*	13.06	—	9.45*	—
Maximum	19.57	24.65	13.06	2.76	10.08	6.85
Minimum	18.34	24.17	12.79	2.65	10.01	5.04
Average	19.00	24.37	12.88	2.70	10.05	6.18

\* Not included in average.

## REPORT ON MOISTURE IN SELF-RISING AND PANCAKE FLOURS, ETC.

By L. H. BAILEY (Bureau of Agricultural Chemistry and Engineering, Washington, D. C.), *Associate Referee*

Self-rising plain flour and self-rising pancake flour differ from most cereal products in that they contain approximately 1.5 per cent of sodium bicarbonate as one of the constituents of the leavening agent present.

Since sodium bicarbonate begins to lose carbon dioxide and water at approximately 50°C., it is evident that the loss in weight obtained by drying self-rising flours in an oven will include some carbon dioxide as well as water.

In order to test the suitability of the ordinary drying methods for products of this type, one sample of self-rising plain flour and two samples of self-rising pancake flours of different manufacture were obtained. These samples were dried in a 70°C. vacuum oven, a 100°C. vacuum oven, a 130° air oven, and a 140°C. air oven supplied with an aluminum plate. Five collaborators made determinations on these samples at 100°C. in a vacuum.

Concordant results were not obtained, hence no values are given in this report.

One collaborator experimented by making a moisture determination on these samples by using a high frequency electric current and obtained some promising results. He ascertained that he did not have his results vitiated by the presence of carbon dioxide. His apparatus, however, will not be immediately available to other laboratories even if he succeeds in working out a suitable method.

Finally the Associate Referee made some moisture determinations on a sample of self-rising flour by the Bidwell-Sterling method of distillation with toluene. The replicate results were in good agreement, and this method may prove to be satisfactory for determinations of this kind; however more work should be done with a variety of samples.

It is recommended that the work be continued and that collaborative results be obtained by the toluol distillation method.

## REPORT ON STANDARD SOLUTIONS

By R. L. VANDAVEER (U. S. Food and Drug Administration, New Orleans, La.), *Referee*

A number of methods on standard solutions, principally solutions of acids and alkali, and permanganate, have been adopted as official by this Association. All of those procedures having this status have been found to be stable for long periods of time under practical conditions of use and storage. Any special precautions necessary to insure stability have been written into the methods.

If a decrease in active strength can be avoided by the inclusion of suitable precautionary statements, the direction for the preparation and standardization should include the methods of preserving the strength of the solution. Even under the best of conditions, certain solutions may slowly lose their strength. This rate of deterioration should be known in order that the chemist using such solutions can be apprised of their behavior and thus can determine the frequency of standardization necessary.

The above criteria as a measure of usefulness of a standard solution have been restated below, along with other fundamental precepts that must be applied to the standard solution before it can be acceptable as such:

(1) The normality or molarity of the standard solution must be accurately determinable by a pure primary standard or by a substance whose active strength is known.

(2) Preferably, the standard solution should be stable during practical conditions of use and storage. If not stable, the rate of deterioration or change in working titer of the solution must be known.

(3) Pure chemicals, with which to prepare the standard solution, or at least chemicals having no active impurities (as, for example, NaCl in sodium thiocyanate), should be available.

#### RÉSUMÉ OF COLLABORATIVE EFFORT

This year reports of collaborative study on the standardization of solutions of (1) iodine and arsenite, (2) sulfuric acid, and (3) silver nitrate and thiocyanate are submitted for consideration. No reports were received on the preparation and standardization of sodium thiosulfate or of hydrochloric acid from constant boiling acid.

There follows a discussion of each associate referee's report on the above-mentioned solutions, with such recommendations by the Referee as would appear appropriate.

*Iodine and arsenite solution.*—The associate referee submitted a solution of sodium arsenite to six collaborators for determination of its normality by means of standard iodine solution, which was to be standardized by the collaborator according to the procedure submitted. The associate referee directs the use of arsenic trioxide obtained from the Bureau of Standards to determine the strength of the 0.1 *N* iodine solution. Thus, the reported results of the collaborators reflect the accuracy of the method studied for the preparation and standardization of iodine solution, and also indirectly prove out the reliability of the procedure for preparing the standard arsenite solution.

The analytical results submitted by the collaborators on the unknown arsenite solution are in close agreement, varying only from 0.09954 to 0.09974, which leaves no doubt as to the accuracy of the associate referee's procedure.

Since the associate referee did not have occasion to study the stability of standard solutions of iodine and arsenite over a period of time, and since

it is well known that light and air, among other factors, may adversely affect iodine solutions upon extended exposure, it is recommended that the proposed method be supplemented to include a caution, which should follow immediately after the directions for preparation of the standard solution, "Solution should be kept in a dark-brown, glass-stoppered bottle, away from light. It should be restandardized as frequently as is necessary."

*Sulfuric acid.*—The Association recommended last year that the tentative method for preparation and standardization of sulfuric acid solutions be subjected to collaborative study. Accordingly, the associate referee prepared a sample solution consisting of approximately 70 per cent sulfuric acid and sent a portion to each collaborator. From the specific gravity of the solution and by the use of the Pickering-Marshall formula, each collaborator prepared a 0.1 *N* solution. Further, each collaborator standardized this 0.1 *N* solution by means of borax—the primary standard used in the official hydrochloric acid method.

Then the collaborators were requested to report the quantity (ml.) of standard sulfuric acid required to neutralize an "unknown" submitted to them. Since the unknown was pure sodium carbonate, the associate referee has given the collaborative results in the last column of Table 1, in terms of "normality," in order to facilitate comparison with the results obtained by means of borax. The method submitted to collaborative study, according to the reported results, yields a solution of a known and accurate strength when measured by means of borax.

It is noted that all six collaborators agreed very closely in their results on the specific gravity of the approximately 70 per cent sulfuric acid submitted to them by the associate referee. Five of the six determined the specific gravity at 25°/25° C. (or at very nearly this temperature), and their results varied only from 1.6100 to 1.6105. While specific gravity results are reported to only the fourth decimal place, some of the collaborators calculated and used specific gravity values to five decimals. This accounts for the differences in grams per liter for Solution A, used by several collaborators where identical specific gravities are shown. However, the collaborators agree to within one part in 1700 as to the quantity of Solution A needed to make one liter of 0.1 *N* acid. These results more than corroborate the reported accuracy of the formula of one part in 1500, *This Journal*, 23, 542 (1940).

*Silver nitrate and thiocyanate solutions.*—The associate referee studied and submitted to collaborative study, three methods for the standardization of silver nitrate solutions, and all the procedures yielded very good results. The Mohr and Volhard procedures are believed to be better known than is the procedure of Fajans, and since the Fajans method offers little if any advantage over the Mohr method, only the Mohr and Volhard procedures are being recommended to the Association.

## RECOMMENDATIONS\*

It is recommended—

(1) That since last year the Association, on the basis of excellent collaborative results, adopted as official (first action) the associate referee's procedure for the preparation and standardization of potassium permanganate solution, this procedure of standardization be now adopted as official (final action).

(2) That the procedure for the preparation of standard hydrochloric acid from constant boiling acid be studied.

(3) That the preparation and standardization of sodium thiosulfate solutions be studied.

(4) That the method presented for standardization of sulfuric acid solutions by means of the specific gravity method be adopted as official (first action).

(5) That the method for preparation of standard arsenic trioxide solutions be adopted as official (first action).

(6) That the method for standardization of iodine solutions, with recommended supplemental precautions, be adopted as official (first action).

(7) That the Mohr and Volhard methods submitted by the associate referee for the standardization of silver nitrate solutions be adopted as official (first action).

(8) That direct methods for the standardization of thiocyanate solutions be studied.

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No report on constant boiling hydrochloric acid was given by the associate referee.

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No report on sodium thiosulfate was given by the associate referee.

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REPORT ON SILVER NITRATE AND THIOCYANATE  
STANDARD SOLUTIONS

By E. C. DEAL (U. S. Food and Drug Administration,  
New Orleans, La.), *Associate Referee*

This year a study was made of various methods for standardization of silver nitrate and thiocyanate solutions, a subject that has not been studied by the Association. The following well-known procedures were selected for collaborative study:

- I. Fajans<sup>1</sup> method with adsorption indicator (fluorescein), direct titration.
- II. Mohr's<sup>2</sup> method with potassium chromate indicator (direct titration).
- III. Volhard's<sup>3</sup> method with ferric indicator (indirect titration).

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 44 (1941).

<sup>1</sup> K. Fajans and H. Wolf, *Z. anorg. allgem. Chem.*, 137, 221 (1924).

<sup>2</sup> *Ann.*, 97, 335 (1856).

<sup>3</sup> *Ibid.*, 190, 1 (1878).

These methods are discussed in detail by Kolthoff.<sup>4</sup>

Preliminary work showed that all three methods give satisfactory results if caution is used in observing end points and proper end-point corrections are applied where necessary. The Fajans and Mohr methods have the advantage of being direct titrations, but their application is limited in that both require practically neutral solutions for titration or, at most, only feebly acid or feebly basic solutions. The Volhard method, while indirect, has the advantage of being applicable to titrations, even in acid media. While these methods generally specify sodium chloride as the primary standard, potassium chloride and potassium bromide were used by the Associate Referee because of their equivalent weights and because they are easily purified. The Fajans method works equally well with either potassium chloride with fluorescein indicator, or potassium bromide with eosin indicator. However, the precipitated silver bromide with adsorbed indicator was found to be extremely sensitive to light. For this reason, and because the major use of the standard solutions is in the determination of chlorides, recrystallized potassium chloride as the primary standard and fluorescein as the adsorption indicator were chosen. Solutions thus standardized may be used for determining any of the halogens except, of course, fluorides.

In order to compare the relative merits of the various methods for the standardization of silver nitrate solutions the three following procedures were studied collaboratively on a solution of silver nitrate prepared according to the method presented.

#### STANDARD SILVER NITRATE SOLUTION

Dissolve slightly more than the theoretical quantity of reagent grade  $\text{AgNO}_3$  (equivalent weight, 169.89) in water (halogen free) and dilute to volume. Have all glassware thoroughly clean, avoid contact with dust, and keep the prepared solution in amber-colored, glass-stoppered bottles away from light.

#### Method I—Fajans

##### REAGENTS

(1) *Pure potassium chloride*.—Recrystallize reagent  $\text{KCl}$  three times from water, dry at  $110^\circ \text{C}$ ., then heat at approximately  $500^\circ \text{C}$ . to constant weight.

(2) *Fluorescein indicator*.—A 0.2% solution of fluorescein in alcohol (or an aqueous solution of the sodium salt).

##### DETERMINATION

Weigh accurately a sufficient quantity of the  $\text{KCl}$  to yield a titration of approximately 40 ml. (about 0.3 gram in the case of 0.1 *N* solution) and transfer to a 250 ml. glass-stoppered Erlenmeyer flask with 40 ml. of water. Add 5 drops of the fluorescein indicator and run in the  $\text{AgNO}_3$  solution with constant shaking until the first permanent rose-pink color is produced in the solution or *on the precipitate*. If the first perceptible color is taken as the end point, the blank is negligible and may be disregarded. Calculate the normality of the  $\text{AgNO}_3$  solution. Equivalent weight of  $\text{KCl}$  is 74.55. Carry out the titration in diffused light.

<sup>4</sup> Volumetric Analysis, Kolthoff and Furman, Vol. II.

## Method II—Mohr

## REAGENTS

- (1) *Pure potassium chloride*.—Prepare as directed in Method I.
- (2) *Potassium chromate solution*.—A 5% solution of reagent  $\text{KCrO}_4$  in water.

## DETERMINATION

Weigh accurately a sufficient quantity of the  $\text{KCl}$  to yield a titration of approximately 40 ml. (about 0.3 gram in the case of 0.1  $N$  solution) and transfer to a 250 ml. glass-stoppered Erlenmeyer flask with 40 ml. of water. Add 1 ml. of the chromate indicator and titrate with the  $\text{AgNO}_3$  solution until the appearance of the first perceptible pale red-brown color. Subtract from the titration the amount of  $\text{AgNO}_3$  solution required to produce the end-point color in 75 ml. of water containing 1 ml. of the chromate indicator. Calculate the normality of the  $\text{AgNO}_3$  solution. (Equivalent weight of  $\text{KCl}$  is 74.55.)

## Method III—Volhard

## REAGENTS AND SOLUTIONS

- (1) *Pure potassium chloride*.—Prepare as directed in Method I.
- (2) *Ferric alum solution*.—A saturated solution of  $\text{FeNH}_4(\text{SO}_4)_2$  in water.
- (3) *Nitric acid* (1 + 1).—Dilute fresh reagent concentrated  $\text{HNO}_3$  with an equal volume of water.
- (4) *Nitric Acid* (2%).—Prepare from fresh reagent concentrated  $\text{HNO}_3$ .
- (5) *Potassium or ammonium thiocyanate standard solution*.—Prepare an approximately 0.1  $N$  solution from reagent-quality chemical that shows no chloride (see "Volumetric Analysis" by Kolthoff and Furman, Vol. II, 1929 Ed., p. 221). Determine the working titer by accurately measuring 40–50 ml. of the standard silver nitrate solution, adding 2 ml. of the ferric alum indicator and 5 ml. of the  $\text{HNO}_3$  (1 + 1), and then titrating with the thiocyanate solution until the solution shows a pale rose coloration after vigorous shaking.

## DETERMINATION

Weigh accurately a sufficient quantity of the  $\text{KCl}$  to yield a titration of approximately 40 ml. (about 0.3 gram in the case of 0.1  $N$  solution) and transfer to a 250 ml. glass-stoppered Erlenmeyer flask with 40 ml. of water. Add 5 ml. of  $\text{HNO}_3$  (1 + 1) and run in an excess of the  $\text{AgNO}_3$  solution. Mix, and allow to stand a few minutes protected from light. Filter through a Gooch crucible prepared with medium pad of asbestos previously rinsed with dilute  $\text{HNO}_3$ . Wash the flask and precipitate with several small portions of 2%  $\text{HNO}_3$ , passing the washings through the crucible until the filtrate and washings measure approximately 150 ml. Add 2 ml. of the ferric indicator and titrate the residual  $\text{AgNO}_3$  with the standard thiocyanate solution. From the titration, together with the ratio of the two standard solutions, calculate the normality of the  $\text{AgNO}_3$  solution. (Errors of a blank are compensating and may be disregarded.) Equivalent weight of  $\text{KCl}$  is 74.55.

## COLLABORATIVE STUDY

A 0.1000  $N$  solution was prepared from silver nitrate that had been recrystallized, dried at  $110^\circ\text{C}$ ., and then fused at  $225^\circ\text{C}$ ., by weighing out the theoretical quantity (101.934 grams per 6 liters) and making to volume at  $20^\circ\text{C}$ . Portions of this solution were sent out to collaborators. A sample, "unknown," was also sent out with instructions for titrating with a thiocyanate solution, the strength of which had been determined by reference





to the silver nitrate solution. This sample was reagent mercuric sulfate that showed no loss of moisture when dried 48 hours over concentrated sulfuric acid.

#### RESULTS BY COLLABORATORS

The theoretical equivalent of sample "X" (mercuric sulfate) is 67.41 ml. of 0.1 *N* thiocyanate per gram. The mean value found by the collaborators was 67.27 ml., indicating a purity of 99.8 per cent. The silver nitrate solution was made up to be exactly tenth normal. The mean value found for the normality of the solution was 0.0999 by the Fajans method, 0.0998 by the Mohr method, and 0.1000 by the Volhard method. If the mean value of the results obtained by the collaborators represents the true normality of the solution, the greatest error of the collaborator whose deviation was greatest would be 0.2 per cent, 0.5 per cent, and 0.3 per cent, respectively, for the three methods. Judged by the submitted averaged results, any normality figure can be expected to deviate not more than 1 part in one thousand. The results show that the deviations from the mean of one collaborator's normalities are considerably greater than the results of the other collaborators, but if his values are excluded the maximum deviation is reduced to 0.2 per cent for the Fajans, 0.1 per cent for the Mohr, and 0.2 per cent for the Volhard method, while the average deviations from the mean are 0.08 per cent, 0.07 per cent, and 0.07 per cent, respectively.

The work this year did not include the direct standardization of thiocyanate solutions but included the preparation of a thiocyanate solution to be standardized indirectly for use in titrating the "unknown" sample. A limited amount of investigation by the Associate Referee shows that the use of purified mercuric sulfate is promising as a primary standard.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That the method of preparation of silver nitrate solutions given and the standardization of solutions of silver nitrate by the Fajans, Mohr, and Volhard methods be adopted as official (first action).
- (2) That the method of determining the strength of thiocyanate solutions by reference to standardized silver nitrate solutions be adopted as tentative.
- (3) That further work be done on methods for the standardization of thiocyanate solutions, and that attention be given to the feasibility of using mercuric sulfate as a primary standard.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 44 (1941).

## REPORT ON STANDARDIZATION OF SULFURIC ACID

By H. W. CONROY (U. S. Food and Drug Administration,  
Kansas City, Mo.), *Associate Referee*

Collaborative study of the Pickering-Marshall method for the preparation and simultaneous standardization of sulfuric acid and for the standardization by borax was carried out as recommended, *This Journal*, 23, 543 (1940), by the methods adopted as tentative and published in *Methods of Analysis, A.O.A.C.*, 1940.

Sample A, consisting of approximately 70 per cent sulfuric acid, was sent to each collaborator with instructions to determine the specific gravity and to prepare 0.1 *N* solution of the acid in accordance with the Pickering-Marshall method.

Each collaborator was asked to standardize the 0.1 *N* sulfuric acid, and to use borax prepared according to instructions.

In addition, the unknown Sample B (consisting of anhydrous sodium carbonate) was submitted to each collaborator, with instructions to titrate the salt with the prepared 0.1 *N* sulfuric acid. The method for carrying out the titration was the same as given, *This Journal*, 22, 103 (1939), "Standardization of Acid Solutions with Sodium Carbonate", with the following exception. The phrase "about 0.21 gram" was substituted for "sufficient anhydrous  $\text{Na}_2\text{CO}_3$  to titrate approximately 40 ml." The collaborators were asked to submit titration values corrected to 20° C. and the weight of salt taken. Table 1 summarizes the collaborative results. The normality against sodium carbonate was calculated by the Associate Referee on the basis of data supplied by each collaborator.

## COMMENTS BY COLLABORATORS

*G. M. Johnson.*—The determination of the specific gravity of the  $\text{H}_2\text{SO}_4$  was made in the same manner as, and compared with, that of water. That is, after the pycnometer is filled at the given temperature, it is allowed to stand 15 minutes before being weighed. When the pycnometer is standardized with water, there is a definite loss due to evaporation, but in the case of the acid there is no loss by volatilization during this time. Consequently, it would seem that the analyst should standardize the pycnometer by weighing as soon as possible after taking it out of the bath rather than waiting 15 minutes. I might suggest in connection with the strength of the  $\text{H}_2\text{SO}_4$  used (70%), that it will pick up moisture in a moderately humid atmosphere. Van Nostrand's Manual gives a table showing the relative vapor pressures of  $\text{H}_2\text{SO}_4$  of different concentrations. That given for 70% acid is 5.2%, which shows that under practically all laboratory conditions at all times of the year, the relative humidity of the air will be appreciably in excess of this figure and consequently will cause absorption of water by the acid if exposed. I would suggest that an acid concentration of about 55% would be more suitable and would be less inclined to absorb water. The relative humidity of this acid at 25° C. is 26.8%.

*Wm. Horwitz and S. H. Perlmutter.*—At the suggestion of L. C. Mitchell the following ammonium sulfate method was used:

Transfer 50 ml. of the acid to a platinum dish, place under a bell jar containing a beaker of strong ammonia, and allow to stand for about an hour. Dry the dish

TABLE 1.—*Collaborative results (all normalities corrected to 20° C.)*

COLLABORATOR	PICKERING-MARSHALL METHOD					
	SPECIFIC GRAVITY "A"		GRAMS/LITER "A" FOR 0.1 N SOLUTION	CALCULATED NORMALITY	NORMALITY AGAINST BORAX	NORMALITY AGAINST Na <sub>2</sub> CO <sub>3</sub>
E. H. Berry Chicago	1.6103	25° C.	7.0000	0.1000	0.1000	0.0999
		25			0.1001	0.0999
					Av. 0.1001	Av. 0.0999
H. W. Conroy Kansas City	1.6100	25° C.	7.0020	0.1000	0.1000	0.0999
		25			0.1001	0.0998
					0.1000	0.0999
					Av. 0.1000	Av. 0.0999
Wm. Horwitz Minneapolis	1.6101	25° C.	7.0018 <sup>A</sup>	0.1000	0.1000	0.1000
		25			0.1001	0.1000
					0.1000	0.1000
					0.1000	0.1000
					0.1000	Av. 0.1000
					0.1000	Av. 0.1000
G. M. Johnson St. Louis	1.6127 <sup>B</sup>	20° C.	D	0.1001	0.1003	0.0997
		20			0.1002	0.1000
					0.1003	0.1000
	1.6130 <sup>C</sup>	20° C.			0.1003	0.1000
		20			0.1003	0.1000
					Av. 0.1003	Av. 0.0999
W. H. King New Orleans	1.6100	25° C.	7.0014	0.1000	0.0999	0.0998
		25				
S. H. Perlmutter Minneapolis	1.6105	24.7° C.	6.9987	0.1000	0.1000	0.1000
		24.7			0.1001	0.1000
					0.1000	0.1000
					0.1000	0.1000
					Av. 0.1000	Av. 0.1000
					Av. 0.1000	
Iman Schurman Cincinnati	1.6101	25° C.	7.0007	0.1000	0.1001	0.0997
		25			0.1001	0.0998
					0.1001	0.0997
	(1.6105)				Av. 0.1001	0.0997

A—Calculated value, 7.0007 grams/liter.

B—Pycnometer weighed after 5 minutes.

C—Pycnometer weighed after 15 minutes.

D—Calculated value, 7.0037 grams/liter, based on sp. gr. 1.6127.

Calculated value, 7.0017 grams/liter, based on sp. gr. 1.6130.

Av. 7.0027 grams/liter.

Actually used 13.9775 grams/2 liters at 32° C. Reduced to 20° C. gives a normality of 0.1001.

and contents at 100° C. in a drying oven overnight, cool, and weigh. Ignite at 500° C., cool, and reweigh. Calculate the normality, assuming the loss in weight is  $(\text{NH}_4)_2\text{SO}_4$ .

	0.1000 (Horwitz)
Normality $\text{H}_2\text{SO}_4$	0.1002 (Perlmutter)
	0.1001 (Perlmutter)

Table 2 summarizes the collaborative results based on each analyst's average normality value.

TABLE 2.—Summary of Table 1 data based on each average result

	METHOD			
	MARSHALL-PICKERING	SODIUM CARBONATE	BORAX	
Range of collaborative results {	Max.	0.1001	0.1000	0.1003
	Min.	0.1000	0.0997	0.0999
Average (Mean)		0.10001	0.10006	0.09989
Mean error of Mean-Dm			0.00005	0.00004
corresponding to an accuracy of (%)			0.05	0.04
Av. deviation			0.00009	0.00008

### DISCUSSION

The Pickering-Marshall method for simultaneous preparation and standardization of sulfuric acid solutions permits duplication of results by different analysts with a high degree of accuracy. A comparison of the calculated weights of Sample A per liter to give a 0.1000 *N* solution shows that the difference between the maximum and minimum values is no more than 0.6 part per thousand. The collaborator whose result was farthest from the average would be in error no more than 0.3 part in a thousand.

If it is assumed that the standard solutions prepared by the collaborators using the Pickering-Marshall method are 0.1000 *N* (in one case 0.1001), then the collaborator whose determination with borax is at greatest variance would be in error no more than 2 parts per thousand. With sodium carbonate the greatest deviation would be no more than 3 parts in a thousand.

### RECOMMENDATIONS\*

It is recommended—

(1) That the tentative methods for standardization of sulfuric acid, *Methods of Analysis*, A.O.A.C., 1940, by the Pickering-Marshall method, and with borax be adopted as official.

(2) That the standardization of sulfuric acid with sodium carbonate be adopted as official.

\* For report of subcommittee A and action by the Association, see *This Journal*, 24, 44 (1941)

Since the method for standardization of 0.1 *N* sulfuric acid in *Methods of Analysis*, A.O.A.C., 1940, page 25, 19(b), involves determination of the normality on the basis of the sulfate content, it is proposed that the method be replaced by the direct methods presented in this report.

The method for standardization of 0.5 *N* sulfuric acid, *Ibid.*, 113, 55, is based upon titration with freshly standardized sodium hydroxide. It is proposed that these instructions be supplemented by the direct methods of standardization given in this report.

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## REPORT ON STANDARD SOLUTIONS OF IODINE AND ARSENITE

By GEORGE M. JOHNSON (U. S. Food and Drug Administration,  
St. Louis, Mo.), *Associate Referee*

It was recommended at the 1939 meeting of the Association that work be done on the method submitted by the previous associate referee for the standardization of iodine solutions, and that standard solutions of arsenite be studied.

The previous associate referee had proposed a method that involves the use of a standard solution of sodium arsenite prepared by weight. It is essentially that given by the National Bureau of Standards for their arsenic trioxide sample No. 63. The present Associate Referee modified these directions somewhat and sent instructions to the collaborators, asking that they prepare 0.1 *N* iodine and arsenite and standardize them. An arsenite solution of a concentration unknown to the collaborators was also sent, with the request that the normality be determined. The methods sent to the collaborators, rearranged and with the changes noted below, are as follows:

### *Arsenite Solutions*

#### REAGENTS

*Arsenic trioxide*.—Use the National Bureau of Standards sample. Dry 1 hour at 105°C. immediately before using.

*Sodium hydroxide*.—Normal solution.

*Sulfuric acid*.—Normal solution.

#### PREPARATION OF STANDARD SOLUTION

Weight the  $\text{As}_2\text{O}_3$  accurately by difference in a small glass-stoppered weighting bottle containing approximately the required quantity (i.e. about 4.95 grams for a liter of 0.1 *N* solution). (It is difficult to brush completely  $\text{As}_2\text{O}_3$  from metallic or glass surfaces.) Dissolve in the normal NaOH solution (50 ml. for each 5 grams of  $\text{As}_2\text{O}_3$ ), in a flask or beaker by heating on a steam bath. Add approximately the same amount of normal  $\text{H}_2\text{SO}_4$ . Transfer quantitatively to a volumetric flask and make to volume. The solution must be neutral to litmus or, at all events, not alkaline. Correct for volume changes due to temperature.

TABLE 1.—*Collaborative results on arsenite solution*

COLLABORATOR	NORMALITY OF IODINE	VOL. OF IODINE USED AT 20°C.	VOL OF UNKNOWN ARSENITE USED AT 20°C.	NORMALITY OF ARSENITE
		ml.	ml.	
Curtis R. Joiner	.09989	39.77	39.89	.09959
New Orleans		39.80	39.89	.09966
		39.78	39.89	.09961
S. B. Falck	.10113	39.40	39.91	.09956
Cincinnati		40.38	40.91	.09955
		41.34	41.89	.09954
E. H. Berry	.09970	39.91	39.91	.09970
Chicago				
J. C. Molitor	.09946	50.09	49.96	.09972
New York				
J. B. Snider	.10150	49.76	50.00	.09952
Minneapolis		49.78	50.00	.09956
G. M. Johnson	.10028	29.75	29.97	.09954
		29.76	29.97	.09958
		29.76	29.97	.09958
Av. .09959				

*Iodine Solutions*

## REAGENTS

*Iodine*.—Analytical reagent grade.*Potassium iodide*.—Analytical reagent grade.*Sulfuric acid*.—(1+10).*Sodium bicarbonate*.—Analytical grade.

## PREPARATION OF STANDARD SOLUTION

Dissolve a weighed quantity of iodine (12.7 grams per liter for a 0.1 *N* solution) and KI in the proportion of 20 grams per 13 grams of I in 50 ml. of water. When the I has dissolved, transfer the solution to a glass-stoppered graduated flask. Dilute to mark with water and mix thoroughly.

## STANDARDIZATION

Transfer an accurately measured portion (40–50 ml. of an approximately 0.1 *N* solution in the case of 0.1 *N* I solutions). Make slightly acid with the  $\text{H}_2\text{SO}_4$ . Neutralize with  $\text{NaHCO}_3$  and add about 2 grams in excess. Titrate with the I solution, using an approximately 0.2% starch solution (5 ml. per 100 ml.) as an indicator. Saturate the solution with  $\text{CO}_2$  at the end of the titration. (This may be accomplished by adding 1 ml. of the dilute  $\text{H}_2\text{SO}_4$  just before the end point is reached.)

From the quantities of I and arsenite solutions used, calculate the titer of the I solution on the basis of the following equation:



The results obtained by the collaborators on the standardization of the unknown arsenite solution are given in Table 1.

#### COMMENTS OF COLLABORATORS

*E. H. Berry.*—It is believed that this procedure is a very accurate method for standardizing iodine solutions.

*J. C. Molitor.*—Difficulty was encountered in dissolving the  $\text{As}_2\text{O}_3$  in 40 ml. of normal NaOH. However it was easily soluble in 60 ml. of normal NaOH.

The solution of arsenite sent to the collaborators was prepared according to the Bureau of Standards method. The normality of the solution at 20°C., based on the weight used, was 0.09969. The collaborative method required 40 ml. of normal NaOH per liter to dissolve the  $\text{As}_2\text{O}_3$  for 0.1 *N* solution. Several of the collaborators had difficulty in effecting the solution although the  $\text{As}_2\text{O}_3$  dissolves fairly readily when heated on the steam bath. The instructions were amended as given above. The directions for preparation of the starch solution sent to the collaborators included detailed directions and required a preservative. As this was not considered necessary more general instructions were included in the method of standardization.

The results obtained by the collaborators are good. The greatest variation from the figure calculated by weight is two parts in a thousand, whereas the average variation is one part in a thousand. There were no difficulties other than those discussed.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method for the preparation and standardization of the iodine solution be accepted as official, first action.

(2) That the method for the preparation of the sodium arsenite solution be accepted as official, first action.

### REPORT ON MICROCHEMICAL METHODS

#### KJELDAHL NITROGEN METHOD

By E. P. CLARK (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.), *Referee*

The extent to which the microkjeldahl method is used for the determination of nitrogen in many classes of compounds would seem to preclude the necessity for a study of the method. However, a survey of the problem reveals an absence of uniformity in both method and apparatus. The procedures are at times inadequate for certain types of compounds or they

\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 44 (1941).





dustrial laboratories, and, with respect to this Association, it has been carefully checked by means of collaborative study. The adoption of this procedure should appreciably aid in standardizing the method as applied to small samples and help to dispel certain prevalent misconceptions concerning its applicability.

#### APPARATUS

The apparatus recommended consists of two parts, the digester (Fig. 1) and the Parnas-Wagner Kjeldahl distilling apparatus (Fig. 2).<sup>1</sup> The distilling ensemble is as simple and easily operated as is consistent with accuracy. A choice should be granted between an electrically or gas heated digester.

The operation of the distillation apparatus is as follows: Steam generated in 1 by a resistance coil immersed in distilled water passes through the trap 2. The quantity of steam delivered is controlled by, preferably, a variable transformer or a rheostat. When 3 and 4 are closed (4 is closed by removing the funnel from a wire hook and allowing it to hang by the rubber connection, thus crimping the tubing), steam passes through the distilling flask 5, thence through the condenser 7, to the collection flask 6. To empty distilling flask 5, the heating current is broken. Immediately the liquid in 5 is emptied into 2. The current is again made; 3 is opened, allowing the liquid in 2 to pass to the waste, and wash water is admitted to 5 through 4; 3 and 4 are then closed and the operation is repeated, which rinses 5, thus conditioning the apparatus for the next distillation.

#### METHOD

The method recommended is the Gunning-Arnold-Dyer modification of combustion with the boric acid method of titrating ammonia. This system is applicable to practically all classes of animal and vegetable materials, pyridine and quinoline derivatives, purines, pyrimidines, amines, amides, oximes, and such substances as carbazole hydrazobenzene and indigotin. By modifying the method according to Friedrich *et al.*,<sup>2</sup> the nitrogen in hydrazines, osazones, and nitro, nitroso, azo, and even certain diazo compounds may be determined with a high degree of precision.

#### PROCEDURE

Approximately 10 mg. of substance weighed upon a 15 × 25 mm. piece of cigarette paper, 40 mg. of HgO, 0.5 gram of K<sub>2</sub>SO<sub>4</sub>, and 1.5 ml. of H<sub>2</sub>SO<sub>4</sub> are placed in a Kjeldahl flask, the dimensions of which are shown in Fig. 1. The flask with its contents is gently heated on the digester until frothing ceases, when the temperature is increased until the mixture vigorously boils and the vapors of the acid rise to within 5 cm. of the mouth of the flask. The total time of digestion should be an hour, and the mixture should be colorless during the latter half of this period. A longer combustion period, for any reason, does no harm. The digest is then cooled, a drop of alcohol is added, and the mixture is again heated until it becomes colorless. When the acid mixture has cooled it is ready for distillation.

With the distillation apparatus in working order and steam having been passed through it for some time, 3 and 4 are opened (the funnel 4 is placed upon the wire

<sup>1</sup> T. K. Parnas and R. Wagner, *Biochem. Z.*, 125, 253 (1921).

<sup>2</sup> A. Friedrich, E. Kühn, and R. Schnüch, *Z. Physiol. Chem.*, 216, 68 (1933).

support in an upright position), the rubber connection between 2 and 5 is closed with haemostatic forceps, and the acid mixture in the digestion flask, diluted with about 8 ml. of water, is transferred through the funnel 4 to 5. The transfer is made quantitatively by rinsing the flask with four 3 ml. portions of water. Before the transfer is made it is expedient to cover the lip of the digestion flask with a thin film of vaseline to prevent the liquid from running down the outside. Sufficient NaOH is added through 4 to neutralize the acid and render the final liquid strongly alkaline. A 40% solution of NaOH containing 5% of crystalline  $\text{Na}_2\text{S}_2\text{O}_4$  is used for this purpose. It is washed into the distilling flask 5 with 2-3 ml. of water, and the system is closed at this point; 3 is then closed, the connection between 2 and 5 is opened, and a small flame is applied to 5. Almost immediately distillation begins. The condensate containing the ammonia is collected in flask 6, containing 2 ml. of 4% boric acid solution and 1 drop of a 0.1% ethanolic solution of methyl red. The distillation is continued with the adapter under the acid solution until 8 ml. of distillate has been collected. The flask is then lowered until the adapter is above the contents of the flask, and the distillation is continued until approximately 1 ml. more of distillate is collected. During this time the outside of the delivery tube is washed with a little water in a fine stream from a wash bottle. The rate of distillation should be so adjusted that the boiling in 5 will not be so violent as to carry over any of its contents mechanically. The condensing water in 7 should also be adjusted so that the temperature of the condensate will not be above 40° C. at the end of the distillation.

The ammonia received in the boric acid solution is titrated with 0.02N HCl, a buret graduated to 0.05 ml. being used.

A blank due to reagents should be determined and subtracted from the buret reading. The percentage of nitrogen in the sample is then calculated as follows:

$$\frac{(\text{ml. of } 0.02 \text{ N HCl used}) (0.28) (100)}{\text{weight of sample}} = \% \text{ nitrogen,}$$

where 0.28 is the number of mg. of nitrogen equivalent to 1 ml. of 0.02 N acid.

#### *Friedrich Method for N-N, NO, and NO<sub>2</sub> Linkages*

Approximately 10 mg. of substance, weighed upon a cigarette paper as previously indicated, is placed in a digestion flask; 1 ml. of constant-boiling hydriodic acid (reagent used in the alkoxy determination)<sup>3</sup> is added, and the mixture is gently refluxed for 45 minutes. More heat is then applied until approximately 0.7 of the hydriodic acid has been slowly distilled from the flask; 0.5 gram of  $\text{K}_2\text{SO}_4$ , 1 ml. of water, and 1.5 ml. of  $\text{H}_2\text{SO}_4$  are added to the concentrate; and the mixture is heated on the digester until most of the water has been removed. After the digest has been cooled, 1 ml. of water is again added and the distillation is repeated. (The purpose of this operation is to remove with steam the liberated iodine, and if this is not accomplished with 2 ml. of water another ml. may be added and the process repeated.) The digest is then cooled and 40 mg. of  $\text{HgO}$  is added, after which the kjeldahlization and distillation are completed in the usual way.

#### DISCUSSION OF METHOD

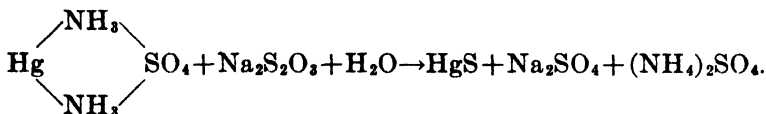
Samples of solid materials, permanent in the air, are most easily weighed upon a tared piece of cigarette paper. This paper, with the sample, is then slipped into the digestion flask with the other reagents and the combustion is started at once.

Much misunderstanding exists as to the time necessary to remove the ammonia quantitatively from the digest after it has been rendered alkali-

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 10, 677 (1938).

line. Experiments conducted to clarify this point have shown that under the conditions of the determination the quantity of distillate recommended to be collected is ample. For example, digests containing 5 mg. of ammonia were distilled at different rates. In all cases the first 4 ml. contained most of the ammonia, the fifth ml. gave a good test (Nessler's reagent), the sixth gave only a faint test, and the seventh was negative.

The object of adding sodium thiosulfate with the alkali is to convert the mercury used as a catalyst to mercuric sulfide. The complex substances formed by mercuric sulfate and ammonia are not readily decomposed by alkali; therefore, if the mercury is not removed, low values are likely to result. Sodium thiosulfate is efficient for this purpose and is convenient when used in the manner indicated. Neuberg<sup>4</sup> gives the following equation for the reaction:



He suggests that the thiosulfate may be used in the solid form in the proportion of 2 parts of crystalline salt to 1 part of mercuric oxide. However, the method indicated is preferable. In neutralizing the digest mixture it is necessary to have an excess of alkali present, but not enough to decompose the mercuric sulfide. When this happens the mixture usually turns yellow and metallic mercury distills into the receiver. During distillation the contents of the distilling flask should always be black from the mercuric sulfide.

The recommended boric acid procedure has been exhaustively tested and has been found to be entirely satisfactory, as many published reports have indicated. Its accuracy is as high as the classical method, and its advantages are that measurement of an excess of standard acid is not necessary, no standard alkali is required, and faint or uncertain end points due to the effect of carbon dioxide are not encountered. In most cases where results have been unsatisfactory they appear to have been due to excessive quantities of boric acid and indicator and attempts to reach a so-called neutral end point. In titrations of this type the end point should be the change in color from the indefinite intermediate neutral range to a distinct pure color formed by an excess acid. Under the conditions of the above procedure 0.01 ml. of 0.01 *N* acid brings this about.

The concept that the Kjeldahl method is inapplicable to many compounds is generally prevalent. At times the idea appears in the literature as a direct statement, but most frequently it is implied in that the nitrogen in many simple compounds is determined by the more cumbersome and time-consuming Dumas method. While the Kjeldahl method may fail, only the cases of certain semicarbazones have come to the Referee's

<sup>4</sup> *Beitr. chem. physiol. Path.*, 2, 214 (1902).

TABLE 1.—*Nitrogen determinations on several types of compounds, where the samples were of the order of 10 mg.*

SUBSTANCE	SAMPLE	0.02 N HCl	NITROGEN
		USED	
	mg.	ml.	per cent
Acetanilide	12.07	4.47	10.38
	10.36	3.86	10.44
	11.18	4.17	10.44
	11.64	4.31	10.37
			Calc'd 10.37
Diphenylamine	9.83	2.90	8.27
	10.03	2.98	8.32
	10.55	3.14	8.34
	11.86	3.52	8.32
			Calc'd 8.28
Phenothiazine	11.62	2.91	7.02
	11.03	2.78	7.06
	9.98	2.49	6.99
	10.44	2.60	6.98
			Calc'd 7.04
Camphor oxime	11.26	3.39	8.44
	11.31	3.41	8.45
	11.20	3.37	8.43
	11.36	3.42	8.44
			Calc'd 8.38
Dihydroisotenulin phenylhydrazone	10.81	2.70	7.00
	9.71	2.42	7.00
			Calc'd 7.03
Dihydrotenulin phenylhydrazone	12.38	3.08	6.96
	11.45	2.83	6.93
			Calc'd 7.03
1-Hydroxy-3, 4-dimethoxybenzene 3, 5-dinitrobenzoyl ester	10.54	3.02	8.06
	10.60	3.05	8.06
			Calc'd 8.04
Atropine sulfate*	11.68	1.45	3.48 a
	10.32	1.43	3.88 b
	10.35	1.48	4.01 c
	10.76	1.52	3.96 d
	9.95	1.38	3.89 e
	10.98	1.53	3.91 f
			Calc'd 4.03
Strychnine hydrobromide*	10.50	2.39	6.38 a
	9.77	2.22	6.37 b
	9.16	2.09	6.39 g
			Calc'd 6.47
Quinine hydrobromide*	9.99	2.23	6.26 a
	10.06	2.33	6.49 b
	11.25	2.62	6.53 g
			Calc'd 6.62

\* These alkaloids were known to contain some moisture. The values are given, however, to show the digestion time necessary to reach a constant value (within experimental error). The digestion periods, in hours, are: a, 1; b, 2; c, 3; d, 4; e, 5; f, 6; g, 16.

attention. Some of the alkaloids and related compounds require longer digestion than is specified in the above procedure, but they do yield their nitrogen to the Kjeldahl method. For example, it has been found that the atropine and quinine types of alkaloids require 2 hours' digestion for complete kjeldahlization and a quantitative yield of nitrogen. In general, however, the Kjeldahl method as here modified has a wider range of usefulness than any other method.

The use of 5-10 mg. samples (Table 1) is based on the assumption that they are weighed upon a balance accurate to 0.02 mg. If such an instrument is not available, the usual analytical balance may be used with equal success, provided samples of about 20 mg. are taken. The procedure and the quantity of reagents are the same except that it is expedient to use 0.04 *N* acid for titration. In Table 2 are some results obtained in this manner, where the samples were weighed upon a Becker No. 8 balance that was in fair condition.

TABLE 2.—*Nitrogen determinations in which samples of the order of 20 mg. were weighed upon a Becker No. 8 balance*

SUBSTANCE	SAMPLE	N/30 HCl USED	NITROGEN
	mg.	ml.	per cent
Acetanilide	20.6	4.57	10.36
	20.6	4.56	10.35
	20.7	4.59	10.36
		Calc'd	10.37
Cinchophane	23.2	2.79	5.61
	22.4	2.72	5.66
	21.1	2.56	5.67
	22.25	2.69	5.64
		Calc'd	5.62
$\gamma$ $\gamma$ -Dipyridyl dihydrate	21.2	6.61	14.55
	20.7	6.44	14.53
	22.7	7.08	14.57
	20.3	6.34	14.58
		Calc'd	14.55
Uric acid	21.8	15.53	33.27
	20.6	14.66	33.24
	20.0	14.24	33.25
		Calc'd	33.33
$\alpha$ -Nitronaphthalene	20.4	3.54	8.09
	20.3	3.50	8.05
	22.1	3.81	8.06
	20.2	3.49	8.06
	21.3	3.68	8.07
		Calc'd	8.05

## COLLABORATIVE REPORT ON THE MICRO AND SEMIMICRO KJELDAHL NITROGEN METHOD

By FRED ACREE, JR. (Bureau of Entomology and Plant Quarantine,  
U. S. Department of Agriculture, Washington, D. C.)

A procedure for the micro and semimicro Kjeldahl nitrogen determinations was submitted for collaborative study by the Referee on Micro Methods. A variety of compounds and samples of 5–20 mg. were analyzed.

The results in Table 1 show the degree of accuracy obtained and, in a measure, the applicability of the method.

In the opinion of the collaborator, the claims for the method made by the referee have been substantiated, insofar as the compounds listed in this report are concerned. The apparatus is simple and inexpensive, and because of the smoothly operating procedure the method lends itself equally well to occasional and routine analyses. The desirability of using the so-called boric acid titration as recommended was verified, and no further comment is required. The procedure in general application is many times as rapid as the classical Dumas procedure, and when augmented by the Friedrich modification it appears to have as wide a range of usefulness.

TABLE 1.—*Nitrogen determinations on several types of compounds*

SUBSTANCE AND SAMPLE NO.	WEIGHT	HCl USED	NITROGEN
	mg.	ml.	per cent
Acetanilide			
1 <sup>a</sup>	19.88	3.49	9.83
2	20.45	3.71	10.16
3	19.61	3.56	10.16
4	21.56	3.88	10.08
5 <sup>b</sup>	20.6	3.87	10.52
6	20.5	3.79	10.35
7	20.6	3.77	10.25
8	21.5	3.98	10.36
9	20.9	3.89	10.42
10	19.2	3.54	10.32
11	19.4	3.59	10.36
12	20.6	3.83	10.41
13 <sup>c</sup>	9.71	3.60	10.38
14	10.75	3.97	10.34
15	12.18	4.50	10.33
16	11.56	4.27	10.33
17	11.66	4.35	10.44
18	10.30	3.82	10.38
19	9.41	3.48	10.36
20	11.26	4.15	10.32

<sup>a</sup> Samples 1–4 and all 10 mg. samples were weighed on a balance sensitive to 0.02 mg. Samples 1–4 were digested for a total of 35 minutes.

<sup>b</sup> Samples 5–12 were weighed on a balance sensitive to 0.1 mg. Samples 1–12 were titrated with 0.04 N HCl.

<sup>c</sup> All 10 mg. samples were titrated with 0.02 N HCl.

TABLE 1.—*Continued*

SUBSTANCE AND SAMPLE NO.	WEIGHT	HCl USED	NITROGEN
	mg.	ml.	per cent
21 <sup>d</sup>	5.035	3.71	10.32
22	5.714	4.20	10.29
23	4.948	3.67	10.38
24	5.473	4.04	10.34
25	6.213	4.55	10.26
26	5.691	4.18	10.30
27	5.023	3.68	10.26
28	5.291	3.92	10.36
		Calc'd	10.37
Palmitamide			
29 <sup>e</sup>	11.65	2.24	5.38
30	10.98	2.07	5.28
31	12.01	2.29	5.35
32	9.81	1.89	5.40
33 <sup>f</sup>	11.20	2.16	5.40
34	9.85	1.92	5.46
35	10.22	1.96	5.38
36	11.29	2.16	5.36
		Calc'd	5.51
Uric acid			
37 <sup>g</sup>	9.88	11.84	33.58
38	10.55	12.65	33.58
39	10.96	13.16	33.62
40	12.14	14.52	33.50
		Calc'd	33.43
5-Carbethoxy-2-keto-6-methyl- diphenyl-1,2,3,4-tetrahydro- pyrimidine			
41	11.80	4.79	11.36
42	11.00	4.22	10.74
43	11.82	4.57	10.83
44	12.00	4.59	10.71
		Calc'd	10.81
Nicotinic acid			
45	10.17	1.07	2.94
46	9.73	1.10	3.17
47	10.34	0.88	2.38
48	10.63	1.07	2.82
		Calc'd	11.42
Camphor oxime			
49	10.30	3.03	8.23
50	10.08	2.97	8.25
51	11.23	3.32	8.30
52	11.93	3.49	8.19
		Calc'd	8.38

<sup>d</sup> Samples 21-28 were weighed on a balance sensitive to 0.001 mg. and were titrated with 0.01 N HCl. Samples 5-28 were digested for 30 minutes after becoming colorless.

<sup>e</sup> Samples 29-32 were digested for a total of 30 minutes.

<sup>f</sup> Samples 33-36 were digested for 30 minutes after becoming colorless.

<sup>g</sup> Samples 37-60 were digested for a total of 30 minutes. Unreported results by Clark show that Samples 45-48 require a much longer period of digestion.



TABLE 1.—*Continued*

SUBSTANCE AND SAMPLE NO.	WEIGHT	HCl USED	NITROGEN
	mg.	ml.	per cent
Phenothiazine			
53	12.22	3.05	6.99
54	11.04	2.74	6.95
55	10.43	2.59	6.95
56	10.54	2.66	7.07
		Calc'd	7.04
Diphenylamine			
57	10.27	2.82	7.67
58	10.74	3.04	7.88
59	10.17	2.78	7.66
60	9.76	2.63	7.55
		Calc'd	8.28
Strychnine HBr			
61 <sup>b</sup>	10.34	2.24	6.07
62	12.03	2.59	6.21
63	11.18	2.57	6.64
64	10.47	2.39	6.39
65	10.19	2.36	6.49
66	12.02	2.73	6.36
67	11.29	2.63	6.53
68	10.42	2.42	6.50
		Calc'd	6.47
Quinine HBr			
69	10.28	1.92	5.23
70	9.82	1.67	4.77
71	9.83	2.08	5.93
72	10.40	1.97	5.30
73	11.24	2.62	4.04
74	11.38	2.39	5.88
75	9.57	2.22	6.50
76	11.07	2.62	6.62
		Calc'd	6.62
Atropine sulfate			
77	11.17	0.72	1.85
78	10.32	0.73	1.98
79	10.21	1.13	3.95
80	11.29	1.17	2.90
81	11.36	1.38	3.40
82	10.46	1.43	3.83

<sup>b</sup> In the order given, consecutive pairs of samples for each of the three alkaloids, 61-84, correspond to digestion periods of 30, 60, 90, and 120 minutes. Samples 61-76 deposited a white solid in the neck of the Kjeldahl flask.

TABLE 1.—*Continued*

SUBSTANCE AND SAMPLE NO.	WEIGHT	HCl USED	NITROGEN
	mg.	ml.	per cent
83	10.12	1.42	3.93
84	10.33	1.50	4.07
		Calc'd	4.03
Acetophenone semicarbazone			
85 <sup>1</sup>	11.47	9.12	22.25
86	10.71	8.39	21.92
87	11.33	9.10	22.48
88	9.43	7.49	22.23
		Calc'd	23.72
Pyrethrolone semicarbazone			
89	10.41	6.40	17.22
90	9.72	5.88	16.95
91	10.19	6.22	17.09
		Calc'd	17.87
$\alpha$ -Nitronaphthalene			
92	10.95	3.12	7.98
93	12.70	3.66	8.09
94	10.14	2.88	7.96
95	12.54	3.57	7.97
		Calc'd	8.05
<i>p</i> -Nitrosodiethylaniline			
96	11.37	6.32	15.55
97	11.05	6.21	15.73
98	10.57	5.98	15.83
99	10.48	5.88	15.72
		Calc'd	15.74

<sup>1</sup> Samples 85-99 were analysed by the Friedrich modification. Samples 96-99 deposited a white solid in the neck of the Kjeldahl flask.

## REPORT ON INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS

### PYRETHRUM, DERRIS, AND CUBE

By J. J. T. GRAHAM (Agricultural Marketing Service,  
Washington, D. C.), *Referee*

In view of the fact that the methods of analysis for pyrethrum, derris, and cube had received considerable study in previous years it seemed unnecessary to request extensive collaboration during 1940.

### PYRETHRUM POWDER

For the determination of the pyrethrins the mercury reduction method, *Methods of Analysis, A.O.A.C.*, 1940, 66, 112-113, and the Seil method<sup>1</sup>

<sup>1</sup> *Soap*, 10, 89 (1934).

were studied. As mentioned in the Referee's report for 1939, the mercury reduction method is applicable only to the determination of Pyrethrin I. For the determination of Pyrethrin II, following determination of Pyrethrin I by this method, a modification of the Seil method was used, *Ibid.*, 67, 114.

In Table 1 these methods are designated Method I for Pyrethrins I and II, and the Seil method is designated as Method II for the same determinations.

The collaborative results are given in Table 1.

TABLE 1.—*Collaborative results on pyrethrum powder*

ANALYST	PYRETHRIN I		PYRETHRIN II	
	METHOD 1	METHOD 2	METHOD 1	METHOD 2
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
J. J. T. Graham	0.20	0.23	0.31	0.31
	0.20	0.23	0.31	0.31
Average	0.20	0.23	0.31	0.31
P. P. Powell, Jr. Auburn, Ala.	0.21	0.24	0.39	0.34
	0.20	0.25	0.43	0.33
	0.20	0.25	0.42	0.33
	0.20	0.25	0.44	0.33
	0.19	0.25	0.43	0.37
Average	0.20	0.25	0.42	0.34

Only two analysts reported on the analysis of the pyrethrum sample. The results for Pyrethrin I are in good agreement by both methods. In the determination of Pyrethrin II there is good agreement among the analysts by Method II, but not so good by Method I.

At the 1939 meeting, Method I for Pyrethrin I was adopted as official, first action, and Method I for Pyrethrin II was adopted as tentative.

#### DERRIS AND CUBE POWDER

No results are reported this year on the methods for analysis of derris and cube powder. Considerable work has been done in previous years, and at the 1939 meeting the Jones-Graham chloroform extraction method for determination of rotenone and a method for ether extract were adopted as official, first action.

#### SUGGESTIONS FOR FUTURE WORK

It is suggested that a study be made of methods for the determination of thallium in ant poisons, and for formaldehyde in seed disinfectants in

which the active ingredient is absorbed in an inert carrier such as Bentonite, talc, etc.

#### RECOMMENDATIONS\*

It is recommended—

(1) That Method I, the mercury reduction method, *Methods of Analysis*, A.O.A.C., 1940, 66, 112–113, for the determination of Pyrethrin I in pyrethrum powder, be adopted as official, final action.

(2) That the crystallization method, *Ibid.*, 64, 110, for the determination of rotenone in derris and cube powder be adopted as official, final action.

(3) That the method for determination of ether extract in derris and cube powder, *Ibid.*, 111, be adopted as official, final action.

#### REPORT ON FLUORINE COMPOUNDS

By C. G. DONOVAN (Agricultural Marketing Service,  
Washington, D. C.), *Associate Referee*

As a result of an extensive collaborative investigation of the determination of total fluorine in insecticides last year, the Association adopted as official, first action, the lead chlorofluoride method, and as tentative, the modified Travers and the distillation-thorium nitrate titration procedures.

These methods were published in *Methods of Analysis*, A.O.A.C., 1940, It is the belief of the Associate Referee that with them, or in certain cases with a slight modification, the insecticide chemist is enabled to determine accurately the percentage of total fluorine in any of the proprietary insecticides.

As shown by the collaborators last year, *This Journal*, 23, 547 (1940), the lead chlorofluoride method is applicable to a larger variety of fluorine samples than is any other method. However, it has been observed that in some cases special precautions should be followed and these should be incorporated in the method. The suggested precautions are:

(1) That in the analyses of silicofluorides that are more or less volatile on heating, for example magnesium and sodium silicofluorides, the combined sample and fusion mixture should be covered with a heavy layer (2 or 3 grams) of the alkali carbonates to prevent the possible loss of fluorine by volatilization before the melt is effected.

(2) That during the washing of the gelatinous zinc precipitate it be returned to the beaker three times instead of once or twice as stated in the method.

(3) That after the lead chlorofluoride is precipitated, it should be allowed to stand overnight in a refrigerator or for 1 hour in an ice bath in order to reduce its solubility in the aqueous solution. This precaution is

\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 45 (1941).

particularly necessary in the summer and in laboratories in which the room temperatures are above normal.

The Association has adopted neither officially nor tentatively a method that is specific for the determination of fluorine as silicofluoride. Often it is desirable to determine both sodium silicofluoride and sodium fluoride in the same product. In the laboratories of the Agricultural Marketing Service the method used is the same as the Travers method for total fluorine, except that no silica and acid are added, and the solution is kept alkaline to prevent the formation of additional silicofluoride from any silica present in the sample. A study of this method is suggested for possible adoption by the Association.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the three mentioned precautions for the determination of total fluorine in insecticides by the lead chlorofluoride procedure be incorporated in the adopted method.

(2) That collaborative study of the adopted and tentative methods for the determination of total fluorine be conducted next year.

(3) That collaborative study of a method for the determination of silicofluoride in a mixture with sodium fluoride be conducted next year.

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For report on pyrethrins, derris, and cube, see the report of the Referee on Insecticides, Fungicides, and Caustic Poisons.

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No report on naphthalene in poultry lice products was given by the associate referee.

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#### REPORT ON SUGARS AND SUGAR PRODUCTS

By R. F. JACKSON (National Bureau of Standards, Washington, D. C.), *Referee*

There is need for further study of confectionery methods. This section should be expanded to include the proven methods of analysis now used by industry. Such studies are especially desirable in view of the new Food, Drug, and Cosmetic Act.

A critical study of the Munson and Walker method for reducing sugars has been made. The details of this investigation are presented in *This Journal* as a contributed paper (see p. 767). The authors recommend that the copper be determined analytically and that Hammond's tables be accepted. Conditions for the acid inversion of sucrose, so as to obtain an invert sugar having the same reducing power as synthetic invert, are also described.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 45 (1941).

No report on maple products was given by the associate referee.

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No report on drying, densimetric, and refractometric methods was given by the associate referee, but he recommended that further study be made of the refractive indices of dextrose and invert sugar solutions.

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No report on polariscopic methods was given by the associate referee.

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No report on chemical methods for reducing sugars was given by the associate referee.

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## REPORT ON ACETYL-METHYL CARBINOL AND DIACETYL IN FOOD PRODUCTS

By JOHN B. WILSON (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

As a result of considerable experimentation during the past two years the Associate Referee has formulated a procedure for the determination of diacetyl that appears to warrant collaborative study.

At first it was believed that diacetyl dioxime (dimethylglyoxime) would be suitable for making a standard solution, but subsequently it was found that this substance dissolves with difficulty in water. Diacetyl monoxime was found to dissolve readily in water and to be easily decomposed by the addition of acid.

A solution of 0.47 gram of diacetylmonoxime was dissolved in water and made up to 1 liter. Various quantities of the solution were treated with proper reagents to form a precipitate of nickel dimethylglyoxime and similar quantities were placed in a distilling flask with acid and the diacetyl distilled.

The method follows:

### METHOD FOR DETERMINATION OF DIACETYL

Place the measured quantity of sample in a flask fitted for steam distillation, dilute to 100 ml. with water, and steam distil, collecting about 100 ml. of distillate in a beaker flask containing 2 ml. of hydroxylamine hydrochloride solution (20 grams +80 ml. of water), 5 ml. of sodium acetate solution (20 grams +80 ml. of water) and 20 ml. of water. Have the end of the condenser dip below the surface of the liquid in the receiver until near the end of the distillation. After 100 ml. has distilled, disconnect the condenser, pour 2 or 3 10 ml. portions of water through the condenser, and add 2 ml. of nickel solution (15 grams of  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$  in water to 100 ml.). Place on a steam bath and evaporate to dryness. Add 10 ml. of water, warm till the salts are dissolved, and let stand 6-24 hours. Filter through a tared Gooch crucible, wash with water and finally with about 20 ml. of alcohol, and dry to constant weight at 100° or 110° C.  $\text{Wt.} \times 0.596 = \text{diacetyl}$ .

The undistilled portions of diacetylmonoxime solution were diluted to about 100 ml. with water, and the same reagents were added as before. The results are given in Table 1.

TABLE 1.—*Precipitation of nickeldimethylglyoxime from diacetylmonoxime*

VOLUME OF SOLUTION	DIACETYL CALCULATED	DIACETYL FOUND DISTILLED	NOT DISTILLED
ml.	mg.	mg.	mg.
5	2.0	2.0	2.3
10	4.0	4.1	4.1
20	8.0	7.7	7.7
25	10.0	10.1	9.9
25	10.0	10.0	10.3
5*	10.0	9.4	
5*	10.0	10.1	

\* These determinations were made at another time with a different solution.

As it is believed that these results warrant collaborative study of this procedure, it is so recommended.

## REPORT ON UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

By F. W. ZERBAN (New York Sugar Trade Laboratory,  
New York, N. Y.), *Associate Referee*

In last year's report to the Association it was recommended that the investigation on the nature of the unfermented reducing substances be continued, and that methods for their determination be studied collaboratively, *This Journal*, 23, 562 (1940).

### NATURE OF UNFERMENTED REDUCING SUBSTANCES

The research on this project is being carried out by Louis Sattler, of this Laboratory. G. T. Reich, of the Pennsylvania Sugar Company, has kindly prepared the necessary raw material on a small-factory scale, following one of the procedures described in the previous report. A 60-Brix solution of refined sugar was inverted by heating with invertase at about 55°C. for 10 weeks. It was then diluted and completely fermented with yeast, and the yeast was filtered off. Part of the filtrate was directly evaporated in vacuo at a low temperature and not treated further. The remainder of the filtrate was clarified with neutral lead acetate and delead with hydrogen sulfide, and the filtrate was concentrated in vacuo at a low temperature. The resulting sirup was separated into an alcohol-soluble and an alcohol-insoluble fraction, and the alcohol was removed by distillation. The three preparations obtained in this manner were only recently received at this Laboratory, and the work on them has not progressed sufficiently for submitting a report.

In the meantime a sample of distillery slop has also been furnished by G. T. Reich, and from this a phenylosazone was obtained, which by its melting point, specific rotation, and mutarotation was identified as the osazone of *d*-pseudofructose (*d*-psicose), previously described by Steiger and Reichstein.<sup>1</sup> This part of the investigation is also being continued, and the results will be published later.

#### DETERMINATION OF UNFERMENTED REDUCING SUBSTANCES

Of the various methods proposed for this determination the Associate Referee decided to study collaboratively that of the Java Sugar Experiment Station,<sup>2</sup> which has been used in Java for over ten years, with only such modifications as were necessary to bring it into line with methods previously adopted by this Association. In Java the reduced copper is determined, without filtration of the precipitate, by titration of the un-reduced copper with potassium iodide and sodium thiosulfate; the difference between the titer found and that of a blank run with water under the same condition is equivalent to the reduced copper. In the present study the Munson and Walker method was selected for the copper determination because this method is now used almost exclusively in the United States for regulatory analyses of molasses.

Three molasses samples were submitted to each of nine collaborators, all of whom have sent in reports. No. 1 was a Cuban blackstrap, No. 2 a Cuban high-test molasses, and No. 3 a refiner's molasses (unfiltered sirup).

The following directions were sent to the collaborators:

(1) In the case of blackstrap molasses (No. 1) and refiner's sirup (No. 3) weigh out 12 grams; in the case of high-test molasses (No. 2), 8 grams. (These quantities correspond to about 6 grams of total sugars.) Transfer the sample to a 500 ml. wide-mouthed Erlenmeyer flask, using in all 75 ml. of water. Add 30 grams of coarsely chopped Fleischmann's baker's yeast (free from starch, obtainable in packages of 1 pound), and mix thoroughly with the molasses solution. Close the flask with a stopper provided with a delivery tube, the other end of which is immersed 1 cm. below the surface of water in a beaker. Place the flask in a water bath or thermostat kept at 30° C., and allow to ferment for at least 4 hours, shaking the flask from time to time. When the fermentation is complete, transfer the contents of the flask quantitatively to a 250 ml. volumetric flask (with molasses that does not foam badly the fermentation can be carried out directly in the 250 ml. volumetric flask). Clarify with 15 ml. of neutral lead acetate solution (20 grams of acetate in 100 ml. of solution), make to the mark at 20° C., add 1 gram of dried Filter-Cel, shake well, and filter, discarding the first few milliliters of filtrate. Deleat the entire filtrate with about 0.5 gram of finely powdered anhydrous potassium or sodium oxalate, add 1 gram of dried Filter-Cel, mix well, and filter again. Determine the copper-reducing power in two 50 ml. portions of the final filtrate by the Munson and Walker method, and report the Cu found in each of these determinations.

Run a blank in exactly the same manner as described, but use water instead of molasses. Determine the copper-reducing power upon two 50 ml. aliquots of the final filtrate, and report the Cu found in each.

<sup>1</sup> *Helv. Chim. Acta*, 19, 194 (1936).

<sup>2</sup> *Methoden van Onderzoek*, 6th ed. (1931), p. 365.



If the final filtrate is not sufficient for two 50 ml. aliquots, repeat the experiment and blank, using twice the amount of molasses, added water, yeast, and clarifying agents specified and dilute after fermentation to 500 instead of 250 ml.

(2) The yeast and lead precipitate occupy such a large volume that it is necessary to correct for it. The Scheibler double dilution method is proposed for this purpose.

Run a second set of determinations and blanks exactly as described under (1), but at twice the dilution specified there. If in the first set you used 12, or 8 grams of molasses, respectively, and diluted after fermentation to 250 ml., dilute the same quantity of molasses in the second set to 500 ml.; if in the first set you used 24, or 16 grams, respectively of molasses, and diluted after fermentation to 500 ml. dilute in the second set to 1000 ml. Determine the copper reducing power in two 50 ml. portions of the final filtrate by the Munson and Walker method, and report the Cu found in each of these determinations and in the corresponding blanks.

If desired, the Lane-Eynon method may be used instead of the Munson-Walker method. But if the quantity of unfermented substances is small, the titer is liable to go beyond 50 ml. with 10 ml. of the Fehling solution.

Those collaborators who are accustomed to determine alcohol yields with a particular strain of yeast are requested to run such tests on the three samples submitted, and to determine the unfermented reducing substances in the fermented solution by the Munson-Walker method, so that the results may be compared with those obtained by the method described previously.

The results of the analyses, corrected for the blanks, are shown in Tables 1 and 2, in terms of milligrams of invert sugar corresponding to the milligrams of copper obtained with 50 ml. of solution, reported by the collaborators. The blanks varied from 0 to 1.0 mg. of copper, not any higher than is usually found in blank tests upon the Fehling solution alone.

To convert the figures shown in the tables into percentage on molasses, those for simple dilution must be divided by 24 in the case of Samples 1 and 3, and by 16 in the case of Sample 2; for the double dilution they must be divided by 12 and 8, respectively.

All the analyses listed in Table 1 were made with Fleischmann's baker's yeast, secured locally by each collaborator. W. W. Searight was unable to obtain this yeast in Havana, Cuba, but the Fleischmann Yeast Company prepared three samples of starch-free baker's yeast specially for him. The moisture content and other characteristics of these special yeasts may have been different from those used by the other collaborators, and for this reason the results found by W. W. Searight are reported separately in Table 2.

A. G. Keller used the Soxhlet volumetric method for the sugar determinations, with methylene blue as internal indicator. In this method the effect of dilution on the copper-reducing power is neglected. In Samples 1 and 3, simple dilution, the titers found were within the range of the Lane-Eynon table, and for the double dilution only slightly beyond this range. If the results are recalculated by means of the Lane-Eynon factors, the averages are 95.5 and 47.4 mg., respectively, for Sample 1, and 96.7 and 50.6 mg., respectively, for Sample 3, somewhat higher than those re-

TABLE 1.—*Milligrams of unfermented reducing substances calculated as invert sugar, after fermentation with Fleischmann's baker's yeast*

ANALYST	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	SIMPLE DILN.	DOUBLE DILN.	SIMPLE DILN.	DOUBLE DILN.	SIMPLE DILN.	DOUBLE DILN.
<i>I. Soxhlet's Volumetric Method, Methylene Blue as Indicator</i>						
A. G. Keller	93.3	47.5	30.8	—	94.3	48.1
Baton Rouge, La.	92.6	47.3	30.5	—	94.0	48.2
Average	92.9	47.4	30.6	—	94.2	48.1
<i>II. Munson-Walker Gravimetric Method—Cu Weighed as Cu<sub>2</sub>O</i>						
Ralph Celmer	130.4	50.6	29.5	12.9	125.4	55.7
Geneva, N.Y.	128.4	50.6	28.6	12.0	127.4	55.7
	115.4		29.1		126.4	
	111.5		29.5		125.9	
Averages	121.4	50.6	29.2	12.5	126.3	55.7
J. K. Dale	116.6	54.3	31.8	16.1	121.4	57.5
Terre Haute, Ind.	116.0	53.0	31.9	15.9	121.2	57.3
Averages	116.3	53.6	31.8	16.0	121.3	57.4
C. F. Snyder	113.1	49.4	27.1	12.6	116.5	54.2
Washington, D.C.	117.5	49.1	27.5	12.6	115.4	53.0
	117.3	50.1	27.4	12.6	116.5	52.6
	114.7	50.4	27.8	13.4	113.2	52.8
		51.5	27.5		114.6	51.2
		52.0	28.4		120.2	
			26.1			
Averages	115.7	47.6	27.4	12.8	116.1	52.9
Grand Averages	118.1	51.1	28.6	13.5	120.3	54.5
<i>III. Munson-Walker Gravimetric Method—Cu Weighed as CuO</i>						
Carl Erb	106.4	47.5	28.6	12.5	112.2	53.0
New York, N.Y.	106.2	46.8	27.7	14.3	112.1	53.0
Averages	106.3	47.1	28.2	13.4	112.2	53.0
C. F. Snyder	102.1	46.0	26.2	12.2	106.9	51.4
Washington, D.C.	105.2	46.5	26.5	12.2	104.7	49.1
	105.1	43.9	25.9	12.0	107.5	49.2
	103.1	46.5	26.6	13.0	103.5	49.0
		47.9	25.7		103.9	47.8
		47.8	27.1		109.2	
			24.9			
Averages	103.9	46.4	26.1	12.4	106.0	49.3
Grand Averages	104.7	46.6	26.6	12.7	107.5	50.4

TABLE 1.—Continued

ANALYST	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	SIMPLE DILN.	DOUBLE DILN.	SIMPLE DILN.	DOUBLE DILN.	SIMPLE DILN.	DOUBLE DILN.
<i>IV. Munson-Walker Gravimetric Method—Cu Weighed after Reduction of Oxide</i>						
W. Bondurant	105.8	48.9	29.7	14.3	117.0	52.1
Reserve, La.	106.2	49.7	30.4	14.4	115.7	53.2
Averages	106.0	49.3	30.0	14.4	116.4	52.7
F. M. Hildebrandt						
Baltimore, Md.	104.9	47.2	32.3	15.6	119.4	51.2
C. F. Snyder	104.4	49.4	25.7	12.0	109.6	51.5
Washington, D.C.	106.9	49.1	26.1	12.2	105.8	50.8
	106.5	50.1	25.7	11.9	109.3	50.0
	104.5	50.4	27.0	12.9	104.9	49.4
		51.5	26.0		107.5	48.4
		52.0	27.4		110.7	
			25.2			
Averages	105.6	50.4	26.2	12.3	108.0	50.0
Grand Averages	105.6	49.8	27.6	13.3	111.1	50.8
<i>V. Munson-Walker Method, Volumetric Determination of Cu</i>						
J. K. Dale	98.6	48.4	30.9	15.0	106.0	53.3
Terre Haute, Ind.	99.4	48.2	30.9	15.6	104.6	53.1
Averages	99.0	48.3	30.9	15.3	105.3	53.2
Carl Erb	94.8	44.2	28.6	13.6	105.8	51.8
New York, N.Y.						
D. J. Smith	96.4	41.6	27.8	13.6	104.3	34.8*
Boston, Mass.	95.3	41.4	29.1	13.1	103.4	34.6*
Averages	95.8	41.5	28.5	13.4	103.9	34.7*
Grand Averages	96.9	44.8	29.5	14.2	104.8	52.7

\* Omitted in grand average.

ported by A. G. Keller. With molasses No. 2, simple dilution, the titer was over 81 ml., well beyond the range of the Lane-Eynon table, and the titer for the double dilution was not determined at all.

Some of the collaborators weighed the copper reduced according to the Munson-Walker procedure as cuprous oxide; others ignited the precipitate and reported as cupric oxide or as metallic copper obtained from cupric oxide. Still other determinations were made by titrating the copper in the precipitate by the new (1940) permanganate method (used by Carl Erb) or by the thiosulfate method (used by J. K. Dale and D. J. Smith).

TABLE 2.—*Milligrams of unfermented reducing substances, calculated as invert sugar after fermentation with specially prepared baker's yeasts*

ANALYST	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	SIMPLE DILN.	DOUBLE DILN.	SIMPLE DILN.	DOUBLE DILN.	SIMPLE DILN.	DOUBLE DILN.
<i>Munson-Walker Method, Volumetric Determination of Cu with Thiosulfate</i>						
W. W. Searight						
Havana, Cuba	88.3	44.6	26.8	14.4	97.4	49.5
Yeast I	87.2	44.0	26.8	14.9	97.4	50.0
Averages	87.7	44.3	26.8	14.6	97.4	49.7
Same	91.6	44.8	27.1	14.5	97.4	49.6
Yeast II	92.0	45.2	26.4	15.0	96.8	49.2
Averages	91.8	45.0	26.8	14.7	97.1	49.4
Same	94.8	46.5	27.7	15.9	95.4	48.2
Yeast III	94.3	46.5	27.3	15.4	94.8	48.2
Averages	94.6	46.5	27.5	15.6	95.1	48.2
Grand Averages	91.4	45.3	27.0	15.0	96.5	49.1

Most of the collaborators reported difficulties due to foaming and violent bumping during the boiling with the Fehling solution, particularly with Samples 1 and 3, which contained large quantities of organic and mineral impurities. This naturally affected the average boiling point of the solutions, and consequently the final results, which show large variations irrespective of the method used for determining the copper in the precipitate. The only way to eliminate this source of error is to carry out the reduction at a definite temperature below the boiling point of the mixed solution, for example, by the method of Quisumbing and Thomas, already adopted by the Association. It is proposed to use this method in further work on the project, because only in this way will it be possible to decide whether the different yeast preparations play any part in the discrepancies noted.

The results further show that weighing as cuprous oxide generally gives the highest results, owing to the inclusion of mineral and organic matter in the precipitate. Ignition to cupric oxide gives generally lower results than weighing as cuprous oxide, owing to the elimination of organic impurities, but the values found are in some cases still too high because of occlusion of mineral impurities, as shown by the volumetric determinations of copper in the precipitate. As would be expected, this contamination is rather pronounced in the final molasses at simple dilution. With the final molasses at double dilution and with the high-test molasses, the error due to mineral impurities is masked by the experimental error. In

further work it will be necessary not only to carry out the reduction with the Fehling solution at a definite temperature, but also to determine the copper in the precipitate, either by electrolysis or preferably by one of the volumetric methods of the Association.

Since the results of this year's work were found to be unreliable, no attempt was made to calculate the volume of the yeast plus lead precipitate from the data for simple and double dilution. Some experiments made with lactose by Scheibler's double dilution method indicate that 30 grams of Fleischmann's baker's yeast occupies a volume of about 25 ml., or 10 per cent of the volume of a 250 ml. flask; this would give a correction factor of 0.9.

F. M. Hildebrandt determined the unfermented reducing substances in the residues remaining after estimating the alcohol yield by the usual distillery method, in all three samples. The results by this method (II), compared with those by the method described in this report (I), were as follows, expressed in per cent of the total sugars present in the original molasses:

Sample No.	Method I	Method II
1	7.6	9.7
2	2.6	2.3
3	8.9	8.0

If the correction factor of 0.9 for the volume occupied by the yeast is applied to the figures found by Method I, the results check very closely with those by Method II for molasses No. 2 (2.34 per cent) and for molasses No. 3 (8.01 per cent), but in the case of molasses No. 1 (6.84 per cent) there is a large discrepancy. This question requires further study.

In conclusion, the Associate Referee wishes to express his sincere thanks to all the collaborators for their willing cooperation.

#### RECOMMENDATIONS\*

It is recommended that the collaborative work on the determination of unfermented reducing substances in molasses be repeated next year, that the method of Quisumbing and Thomas be used for the copper reduction, and that the copper in the precipitate be determined volumetrically.

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No report on sucrose in molasses was given by the associate referee.

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No report on honey was given by the associate referee.

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No report on refractive indices of sugar solutions was given by the associate referee.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 62 (1941).

## REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

Four imitation raspberry flavors were prepared, and  $\beta$ -ionone was determined by several chemists, all but one of whom acted as collaborators last year. Flavor 1 was made by diluting remaining portion of Flavor 1 used in last year's work. This flavor contained about 18 per cent by volume of alcohol, extractive matter from black raspberries and from partially fermented black raspberries, and a known quantity of added  $\beta$ -ionone.

Flavor 2 was prepared from Sample 2 used in last year's work with a further addition of  $\beta$ -ionone. It contained about 13 per cent by volume of alcohol, extractive matter from red raspberries, amyl acetate, and added  $\beta$ -ionone. These samples were analyzed by the method, " $\beta$ -ionone in raspberry flavors," published in *Methods of Analysis, A.O.A.C.*, 1940, 332, 70.

TABLE 1.—*Determination of  $\beta$ -ionone in imitation raspberry flavors*

ANAL. FILE NO. PORTION USED	1		2		3	4
	300 ML.	500 ML.	300 ML.	500 ML.	5 ML.	5 ML.
	mg.	mg.	mg.	mg.	mg.	mg.
Calculated	16.2	27	37.2	62.0	102.0	21.6
Collaborator			$\beta$ -ionone	found		
I. Schurman	15.0	lost <sup>a</sup>	33.7	57.5	101.6	20.3
Cincinnati					101.0	19.6
J. Fitelson	14.1	22.9	39.3	64.4	101.8 <sup>b</sup>	19.8 <sup>b</sup>
New York					101.1	19.6
H. W. Gerritz	12.2 <sup>c</sup>	22.8	34.5	56.8	100.6	19.6
San Francisco					99.8	20.0
M. J. Gnagy	— <sup>d</sup>	— <sup>d</sup>	— <sup>d</sup>	— <sup>d</sup>	99.8	17.5
Los Angeles					100.0	18.1
J. B. Wilson	12.3	22.6	39.1	59.5	99.0	20.2
	16.5	22.3			99.5	19.8
Av.	14.0	22.0	36.6	59.6	100.4	19.5

<sup>a</sup> Schurman had had no previous experience with the method, but his results are in line with those of other chemists who collaborated last year. This particular determination was lost due to the bumping and boiling over of the ether solution during the final evaporation. The result obtained was 19.4 mg.

<sup>b</sup> Fitelson noticed that the first precipitates reported on Flavors 3 and 4, which had stood overnight before the final addition of water, were darker and slightly heavier than the second precipitates, which had stood only 2 hours before the final addition of water. He repeated determinations on Flavor 4, obtaining 19.7 mg. when the addition of water was delayed 24 hours and 19.2 mg. when water was added after 2 hours.

<sup>c</sup> Gerrits stated that the 300 ml. portion of Sample 1 was distilled in an apparatus fitted with rubber stoppers instead of ground-glass connections.

<sup>d</sup> Gnagy was unable to obtain results upon Samples 1 and 2 due to the formation of tar-like material in the precipitation flask, which prevented the transfer of the precipitate to the crucible. No other collaborator reported this difficulty.

Flavors 3 and 4 were analyzed by the method,  $\beta$ -ionone (applicable to pure solutions of 100 mg. or less in 5 ml. of alcohol, *Ibid.*, 68. Other flavoring ingredients of non-aldehydic and non-ketonic character, recommended for use in commercial imitation raspberry flavors, were added to Flavors 3 and 4 to test the efficacy of the method in the presence of possible interfering agents.

Flavor 3 contained 0.75 ml. of amyl acetate and 864 mg. of  $\beta$ -ionone in 200 ml.

Flavor 4 was made up of the following ingredients:

$\beta$ -ionone.....	4.08 g.
	ml.
Amyl butyrate.....	1.25
Methyl heptine carbonate.....	0.30
Strawberry aldehyde.....	0.50
Alcohol.....	q. s.
	<hr/>
	200

The results obtained are given in Table 1.

The Referee had difficulty similar to that of Gnagy with a few commercial samples of raspberry flavor that contained substantial amounts of fruit extractives and small quantities of ionone, but is unable to point out the cause of the difficulty. In one or two instances, the flask and crucible were dried in an oven and washed two or three times with small quantities of petroleum benzin, after which the precipitate was easily transferred to the crucible by the dilute alcohol wash solution. In other cases, a distillate from a second portion of the same sample presented no difficulty in transferring. The tarry material is not generated from ionone and the reagents at steam bath temperature, as the Referee has frequently permitted solutions to remain on the bath for 10 minutes or more after all the liquid was gone and had no difficulty with the filtration.

The darkened color of the precipitates noticed by several collaborators appears to be the result of having other flavoring ingredients in the solution.

The results are considered satisfactory, and the Referee is recommending that both methods be made official.

Curl and Nelson, *This Journal*, 22, 684 (1939), report that standard vanillin solution used in the official colorimetric method increases in apparent strength as much as 19 per cent over a freshly prepared solution. The Referee has on hand a standard vanillin solution that has been in use for the past 15 years without apparent change. This standard was prepared by dissolving 1 gram of vanillin in 250 ml. of alcohol and diluting to 1 liter with water. When determinations are to be made, 10 ml. of the solution is diluted to 100 ml. with water, which gives the proper strength for use in the official colorimetric method. The Referee's standard was

compared recently with a newly prepared standard giving readings as follows:

New standard	Old standard
20	20.1
	19.9
	19.9
	20.0
	20.0
Av.	20.0

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method for  $\beta$ -ionone, *Methods of Analysis, A.O.A.C.*, 1940, 332, 68, be adopted as official.

(2) That the method for  $\beta$ -ionone in Raspberry Flavors, *Ibid.*, 69, 70, be adopted as official.

(3) That the following directions for a more permanent standard vanillin solution be added under reagents, *Ibid.*, 321, 6:

*Stock solution of vanillin.*—Dissolve 1 gram of vanillin in 250 ml of alcohol and dilute to 1 liter with water.

*Standard vanillin solution.*—Dilute 10 ml. of the stock solution of vanillin to 100 ml. with water (should be freshly prepared for use in vanillin determination).

(4) That the method for benzaldehyde in beverages (non-alcoholic) and concentrates, *Ibid.*, 148, 29, be adopted as official (first action).

(5) That the method for gamma-undecalactone in beverages (non-alcoholic) and concentrates, *Ibid.*, 149, 30, be adopted as official (first action).

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No report on glycerol, vanillin, and coumarin was given by the associate referee.

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No report on organic solvents in flavors was given by the associate referee.

#### REPORT ON MEAT AND MEAT PRODUCTS

By R. H. KERR (U. S. Bureau of Animal Industry,  
Washington, D. C.), *Referee*

Further work was done with quantitative methods proposed for the determination of dried skim milk and soybean flour in meat and meat products, but the project was not advanced to the stage of sending out samples for collaborative work. It is thought possible that a method for

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 64 (1941).



the quantitative determination of lactose in meat food products that will afford a ready means of estimating dried skim milk may be ready for collaborative work during the coming year and for presentation at the next meeting of the Association. Some study of the possibilities of the Hendrey method proposed at the last meeting for the quantitative estimation of soybean flour was made. This method in reality determines hemi-celluloses, a class of compounds by no means peculiar to soybean flour. The studies that have been made indicate the need for much more extensive work before such a quantitative method can be presented for collaborative work. Therefore it is recommended that work on methods for the quantitative determination of lactose and soybean flour be continued.

Recommendation is made for such action as may be necessary to validate the change in the method for creatin, *Methods of Analysis*, A.O.A.C., 1940, 381, Section 32. This change in the method relates to the substitution of creatinin zinc chloride for potassium bichromate in making the standard solution for color comparison. The change was recommended simultaneously to the Committee on Editing Methods of Analysis and to the Association. The recommendation made last year was accepted by the Editorial Committee, so that the method appears in its modified form in the latest edition of *Methods of Analysis*, but it met with adverse action by Subcommittee C on Recommendations of Referees. Inasmuch as the method results in the conversion of creatin to creatinin, which is then determined by means of the color resulting from its reaction with picric acid in alkaline solution, it seems unnecessary to present supporting data on a comparison of solutions containing unknown amounts of creatinin with solutions containing definite and accurately known amounts of that substance. The amount of potassium bichromate specified in the method as it formerly appeared was in fact established by color comparison with solutions containing definitely known amounts of creatinin. At the time it was originally adopted, no convenient pure preparation of creatinin was readily available. Since creatinin zinc chloride is now readily available in a high state of purity, there seems to be no good reason to retain the obsolete standard in an official method. Accordingly, it is recommended\* that the Association take such action as may be necessary to make the method that now appears in the 1940 edition of *Methods of Analysis*, A.O.A.C., 1940, official.

It is also recommended that the Referee on Meat and Meat Products be relieved of responsibility for the methods of arsenic, copper, and zinc in gelatin, Chapter XXVIII, Sections 64 to 68, inclusive, and that these be assigned to the Referee on Metals in Foods, where the responsibility for these methods belongs.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 58 (1941).

An abstract of the paper, entitled "Experiences of the Massachusetts State Department of Public Health with the Hendrey Method for the Determination of Soy Bean Flour in Sausages," which was presented at the 1940 meeting, will be found on p. 799.

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## REPORT ON SPICES AND CONDIMENTS

By SAMUEL ALFEND (U. S. Food and Drug Administration,  
St. Louis, Mo.), *Referee*

The Association recommended for this year various studies on vinegar, salad dressings, prepared mustard, and spices. This work was assigned to the Referee and four associate referees.

### ASH

Work on this subject was undertaken by the Referee.

There is no record of work on ash in spices since 1915, when Brinton (1) reported to the Association on extensive personal and collaborative work, results showing that the procedures used in different laboratories varied widely, and results were erratic, even under the most favorable conditions. Sindall, as Associate Referee in 1915 (2), submitted collaborative results showing maximum variations of 2.74 per cent for the ash of marjoram, 2.80 per cent for sage, 1.65 per cent for savory, and 1.20 per cent for thyme. The Referee concluded the subject should be studied further, with emphasis on the influence of temperature of combustion and an effort to fix definitely the optimum temperature. Apparently no further studies were undertaken.

Brinton emphasized the need for adhering to the official method by keeping the temperature as low as possible, "below visible red." The present official method refers to the method for ash in grain and stock feeds, which specifies "a low red heat, not exceeding dull redness." Last year the Referee proposed modification of the method by specification of a temperature of 500°C. The Association directed, instead, that "studies be made . . . with a view to fixing the temperature of ignition."

Much of the previous work has been limited to study of a small number of spices. Spices vary greatly in composition. Ash values may range from 2 to 16 per cent, volatile oil from a trace to 20 per cent; and starch, fixed oil, silica, sulfur, and other constituents differ markedly in different spices. A general method must therefore be studied on many different spices. The Referee studied the ashing of the following 18 spices: black pepper, cinnamon, marjoram, thyme, nutmeg, mace, sage, allspice, yellow mustard flour, ginger, cloves, paprika, aniseed, caraway seed, turmeric, cumin seed, cardamon seed, and savory. This selection is believed to be representative of the various types of spices. The general procedure was to ignite the spice, heat in a controlled muffle at 500°C. until no more weight was lost,

determine by wetting down whether any carbon remained, and heat at 550°C. in similar fashion, then at 600°C. and even higher temperatures when necessary.

The muffle heating chamber was approximately 6 inches high, 8 inches wide, and 14 inches deep. The heating elements were exposed at top and two sides. The dishes rested on a silica plate, which completely covered the refractory bottom of the chamber. The thermocouple junction was 2 inches from the top, and some 6 inches from the back. The dishes, heated four at a time, were grouped evenly around the junction. The muffle was thermostatically controlled; the controller had been calibrated by the National Bureau of Standards. The temperature lag in the center of the chamber at 525°C. was somewhat less than 10°. The controller was therefore set 10° lower than the desired maximum temperature. During heating periods the peephole in the door was left open.

The platinum dishes used were flat-bottomed, of 100 ml. capacity, and fitted with aluminum lids, according to Wichmann's design (3). Individual desiccators were used for cooling the dishes. Two types were used—glass ones containing fresh sulfuric acid and aluminum ones with ground flanges, containing anhydrous magnesium perchlorate. The temperature of each dish was determined with a thermometer to insure that it was that of the balance (Blade (4) has demonstrated that a difference in

TABLE 1.—Ash values for spices showing relative efficiency of desiccators

SPICE	ASH		
	FIRST COOLING IN ALUMINUM-Mg(ClO <sub>4</sub> ) <sub>2</sub> DESICCATOR	SECOND COOLING IN GLASS-H <sub>2</sub> SO <sub>4</sub> DESICCATOR	THIRD COOLING IN ALUMINUM-Mg(ClO <sub>4</sub> ) <sub>2</sub> DESICCATOR
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Black pepper	4.76	4.77	4.76
Cinnamon	3.88	3.88	3.88
Marjoram	10.73	10.71	10.72
Thyme	7.45	7.44	7.44
Nutmeg	2.14	2.14	2.14
Mace	2.04	2.04	2.04
Sage	7.79	7.76	7.76
Allspice	4.57	4.58	4.58

TABLE 2.—Gain in weight of ash (%) when left open in Mg(ClO<sub>4</sub>)<sub>2</sub> desiccator for 15 minutes

BLACK PEPPER	CINNAMON	MARJORAM	THYME	NUTMEG	MACE	SAGE	ALLSPICE	AV.
0.07		-0.02*	0	0.05	0.06	0.03	0.02	
0.02	0	0	-0.04	0.05	0.01	0.04	0.03	
0.03	0	0.01	0.01					0.025

\* Minus sign indicates loss of weight.

temperature between dish and balance pan of 5° may cause a weighing error of 1 mg. in a porcelain crucible).

To test the relative efficiencies of the two types of desiccator, ashes of eight different spices that had been heated at 550° until they lost no more weight were heated at 550° for 15 minutes, cooled in individual aluminum-magnesium perchlorate desiccators in open dishes for 30 minutes, and weighed. They were then re-heated at 550° for 15 minutes, cooled in individual glass-sulfuric acid desiccators for 30 minutes, and weighed. They were again heated for 15 minutes and cooled in aluminum desiccators. The results in Table 1 indicate that for this purpose the two types of desiccator are equally satisfactory.

TABLE 3.—*Gain in weight of ash (%) when exposed to air for 15 minutes*

BLACK PEPPER	CINNAMON	MARJORAM	THYME	NUTMEG	MACE	SAGE	ALLSPICE	AV.
0.23	0.09	0.08	0.08					
0.17	0	0	0.01					
0.21	0.01	0.01	0.03	0.06	0.05	0.01	0.03	
0.06	0.05	0.05	0.05	0.06	0.09	0.02	0.07	0.063

To determine whether the covers for the ashing dishes were necessary or advantageous, ashes from eight spices were cooled in a barium perchlorate desiccator in covered dishes and weighed. The dishes were then replaced in the desiccator for 15 minutes with the lids removed, after which the lids were replaced and the dishes were weighed. Several trials were run on each spice. The average gain in weight (Table 2) was 0.025 per cent.

The same eight spices were tested for hygroscopicity of ash by exposing to room atmosphere for 15 minutes ash samples that had been cooled in desiccators in open dishes. The average gain in weight was 0.06 per cent, the black pepper ash being most markedly hygroscopic (av. 0.17 per cent) and the sage ash least so (av., 0.015 per cent).

Brinton (1) states, "After moistening the ash with water the weight before moistening is not regained at the temperature of combustion, even after a reheating of over one hour," and presents figures on which this conclusion is based. His ashing temperature was 400°C. To test this conclusion, the Referee wetted the carbon-free ashes from a number of spices, dried the ash dishes on a steam bath, and heated them for different lengths of time, at 500° or 550°C. This work was incidental to some other tests, and was not planned in a manner to allow clear-cut conclusions, as may be seen from the results in Table 4. Of the 35 tests, 15 ash samples gained an average of 0.04 per cent, 15 lost an average of 0.04 per cent, and 5 samples showed no change. Only one sample showed a marked increase in weight when heated at 550° for 30 minutes. The possibility of contamina-

TABLE 4.—*Change in weight of ash (%) after wetting and subsequent heating*

SPICE	CHANGE IN WEIGHT*							
	HEATED AT 500°C FOR—					HEATED AT 550° C. FOR—		
	5 MIN.	15 MIN.	30 MIN.	45 MIN.	60 MIN.	5 MIN.	30 MIN.	50 MIN.
Black pepper	-0.05						+0.15	
Cinnamon	-0.05	-0.06					+0.04	
Marjoram	-0.03						-0.06	
Thyme	-0.03						-0.06	
Nutmeg			-0.01†		-0.04	0		
Mace			+0.02†		0	+0.01		
Sage			-0.01†		0	+0.02		
Allspice			-0.01†		-0.04	0		
Mustard								-0.01
Ginger				+0.01	-0.03			0
Cloves				+0.01	+0.01			+0.02
Paprika								-0.06
Aniseed					+0.06			
Caraway					+0.07			
Turmeric					+0.06			
Cumin					+0.06			
Cardamom					+0.02			
Savory					+0.03			

\* Minus sign denotes a loss in weight, plus sign a gain.

† Moistened with alcohol.

TABLE 5.—*Change in weight of carbon-free ash (%) on continued heating*

SPICE	CHANGE IN WEIGHT OF ASH*			
	HEATED AT 500°C. FOR—		HEATED AT 550° C. FOR—	
	1 HOUR	2 HOURS	1 HOUR	2 HOURS
Cinnamon	0	0		
Black pepper	-0.02	-0.03		
Thyme	-0.01	-0.01		
Marjoram	-0.04	-0.02		
Cloves	0	0		
Caraway	-0.01	0		
Nutmeg			+0.01	0
Mace			+0.01	-0.01
Sage			-0.02	-0.03
Allspice			0	+0.01
Ginger			0	0
Paprika			+0.01	-0.02
Aniseed			-0.02	-0.04
Turmeric			-0.01	-0.02
Cumin			0	-0.02

\* Minus sign denotes a loss in weight, plus sign a gain.

TABLE 6.—*Spices yielding carbon-free ash at 500°C.*

SPICE	HEATING TIME AT 500°C.	NO. TIMES WETTED DOWN WITH—		LOSS AT 550°C.	LOSS AT 600°C.
		ALCOHOL	WATER		
	<i>minutes</i>			<i>per cent</i>	<i>per cent</i>
Cinnamon	60	0	0	-0.02*	0.80
Black pepper	150	1	1	-0.02	0.10
Thyme	150	1	1	0.04	0.94
Marjoram	150	1	1	0.06	1.17
Cloves	75	0	1	0.03	0
Caraway	125	0	1	-0.01	0.22
Ginger	170	0	1	0	0
Cardamom	125	0	1	0.04	0.16

\* Minus sign denotes gain in weight.

TABLE 7.—*Spices yielding carbon-free ash at 550°C.*

	HEATING TIME AT—		NO. TIMES WETTED DOWN WITH—		LOSS AT 600°C.
	500°	550°	ALCOHOL	WATER	
	<i>minutes</i>	<i>minutes</i>			<i>per cent</i>
Nutmeg	200	30	1	2	0.04
Mace	200	30	1	2	0
Sage	200	30	1	2	1.00
Allspice	200	30	1	2	0.23
Paprika	170	50	0	2	0.10
Aniseed	185	60	0	2	0.29
Turmeric	185	60	0	2	0.22
Cumin	185	60	0	2	0.43
Savory	125	30	0	2	0.51

tion can not be ruled out. In spite of this one result, it is concluded that a heating period of 30 minutes at 550° after the ash is wetted down is reasonably safe.

No consideration has been given in this report to what constitutes "ash" (see Wichmann's extensive discussion (3)). A pertinent question was whether to take as the ash the residue remaining as soon as the free carbon had been driven off at the lowest possible temperature, or whether this carbon-free residue should be heated at that temperature until equilibrium had been established among oxides, carbonates, sulfates, etc.

Nine spice samples were heated at 550°C. until free from carbon, as determined by wetting with alcohol. Six others were heated at 500°C. until free from carbon. The ashes were cooled, weighed, and heated at the original ashing temperature for two consecutive 1-hour periods, with cooling and weighing between the two periods. From the inconsiderable changes that resulted from continued heating (Table 5) it is concluded that it is advisable to cease heating as soon as all the free carbon is gone.

Another question is whether it is not better to use the highest temperature compatible with retaining inorganic constituents, rather than the lowest temperature required to burn off carbon, in order to reduce the ashing time. If there are a number of spices whose ashes, obtained at 500°C., do not lose weight when heated at 550°C., it would be advantageous to heat the spices at the higher temperature from the start. Tables 6 and 7 present the pertinent data on this subject.

Of the 18 spices tested, 8 showed no material loss in the carbon-free ash between 500° and 550°C. Nine others required a temperature of 550°C. to attain a carbon-free ash. Nutmeg, mace, ginger, and cloves showed no loss in ash between 550° and 600°C. Black pepper and Spanish paprika ashes lost only 0.10 per cent between 550° and 600°C. Marjoram, sage, thyme and cinnamon, on the contrary, suffered considerable losses.

Mustard flour did not yield a white ash at any temperature up to 700°. Several procedures were tried to get a carbon-free ash. A large excess of powdered silica, used to increase the surface, yielded an ash apparently free from carbon at 600°C. This was very light and powdery, and difficult to handle without loss. The use of alcohol and glycerol did not help. Magnesium nitrate solution hastened the combustion, but did not solve the problem. Addition of nitric acid was found to be the best means for obtaining a carbon-free ash. It is recognized that this converts carbonates to oxides, and perhaps affects chlorides and sulfates. This may be kept to a minimum by leaching out soluble ash constituents before adding nitric acid. If a constant figure for ash may be obtained by this empirical treatment, it is worth while.

An attempt was made to shorten the ashing time or lower the temperature by introducing oxygen into the combustion chamber during the ashing. The muffle furnace was so large that sufficient oxygen to be effective could not be introduced. It would be interesting to ignite smaller quantities of spice in a small chamber in an atmosphere of oxygen.

The Referee recalls that when he received his first instructions in the general procedures of food analysis, he was cautioned not to use platinum dishes to ash foods with acid residues. Spices were specified among these foods. In this work platinum dishes were used throughout. The dishes were not affected by any of the 18 spices up to 550°. At 600° some of the spice ashes left a light stain, which was readily removed by rinsing the dish with hot nitric acid and heating to a bright red color. The low heat capacity and other advantages of platinum make it eminently the material of choice for ashing spices.

The method sent to collaborators was based on the findings reported above. It was tested on ground samples of black pepper, marjoram, cloves, and yellow mustard flour. The results are given in Table 8.

With the exception of one collaborator's returns, the agreement is quite good. Particularly gratifying are the results on marjoram, a high-ash spice

TABLE 8.—*Collaborative results on ash in spices (%)*

ANALYST	ASH			
	BLACK PEPPER	MARJORAM	CLOVES	YELLOW MUSTARD FLOUR
1	4.71	12.50	5.65	3.76
	4.71	12.50	5.64	3.74
				3.74
2	4.55	12.45	5.58	3.54*
	4.58	12.64	5.60	3.57*
		12.31*		
3	4.84	12.50	5.72	3.78
	4.81	12.49	5.73	3.77
4	4.71	12.51	5.66	3.75
5	4.66†	12.51†	5.66†	3.74†
	4.70‡	12.55‡	5.67‡	
6	4.75		5.62	3.71
7	4.75	12.51	5.76	3.74
	4.75	12.48	5.73	3.73
8	4.76			
9	4.74	12.62	5.59	3.74
			5.59	3.79
Average	4.71	12.52	5.66	3.75
Max.	4.84	12.64	5.76	3.79
Min.	4.55	12.31	5.58	3.54
Standard deviation	0.08	0.06	0.06	0.02

\* Not included in calculation of average and standard deviation.

† Covered dishes.

‡ Uncovered dishes.

known to yield irregular results in the past (1) and on mustard flour, which is difficult to ash.

On the basis of this study, the Referee believes the wording of the ash method should be changed to conform to the instructions guiding this year's collaborative work, which are as follows:

#### ASH

Weigh accurately approximately 2 grams of spice in flat-bottomed dish, preferably of platinum. Place dish in entrance of open muffle so that sample fumes off without catching fire. Place dish in muffle kept at 550° C. for 30 minutes, break up ash with several drops of water, evaporate carefully to dryness, and heat in muffle for 30 minutes. If previous wetting showed ash to be free from C, remove dish to desiccator



containing fresh efficient desiccant ( $\text{H}_2\text{SO}_4$  and anhydrous  $\text{Mg}(\text{ClO}_4)_2$  are satisfactory), allow to cool to room temperature and weigh soon after. If first wetting showed C, repeat wetting and heating until no specks of C are visible, then heat for 30 minutes after disappearance of C. If C persists, leach ash with hot water, filter through quantitative filter paper, wash paper thoroughly, transfer paper and content to the ashing dish, dry, and ignite in muffle at  $550^\circ$  until ash is white. Cool dish, add filtrate, evaporate to dryness on steam bath, and heat in muffle for 30 minutes. Cool, and weigh as directed previously.

(Nutmeg, mace, ginger, and cloves may be heated at  $600^\circ$  without appreciable loss of ash constituents.)

*Ground mustard or mustard flour.*—Ignite as directed previously and heat for 30 minutes at  $550^\circ$ . Leach ash with hot water, filter, and wash thoroughly. Transfer filter paper and contents to ashing dish, dry, and heat in muffle for 30 minutes. Remove dish, allow to cool, add 5–10 drops of  $\text{HNO}_3$ , evaporate to dryness, and heat in muffle for 30 minutes. Repeat  $\text{HNO}_3$  and heating treatment until residue is white. Add filtrate, evaporate to dryness, and heat in muffle for 30 minutes. Cool, and weigh as above.

The collaborators were cautioned to exercise care to avoid loss in ash material, to make weighings with dishes exactly at balance temperature, to expose ash to the atmosphere for the shortest possible time, to get rid of all carbon, to maintain close temperature control in the furnace, and to use a fresh desiccant in a small desiccator. They were requested not to use a furnace that did not have a thermocouple, and preferably to use automatic temperature control.

#### COMMENTS OF COLLABORATORS

*S. D. Fine, St. Louis (No. 4).* Covered Pt dishes, glass desiccator containing  $\text{H}_2\text{SO}_4$ , one dish to a desiccator. Cooling time 30 minutes.

*F. M. Garfield, St. Louis (No. 5).* Pt dishes used. All samples under I were in closed dishes, samples under II were in open dishes. Closed dishes were kept in Al desiccators with  $\text{Mg}(\text{ClO}_4)_2$  desiccant. Open dishes were held in glass desiccators with sulfuric acid as desiccant, one dish per desiccator. Time of cooling for all dishes was 25 minutes.

*F. J. McNall, Cincinnati (No. 7).* Flat-bottomed 100 ml., open platinum dishes were used for ashing. Individual 6" glass desiccators using  $\text{H}_2\text{SO}_4$  for a desiccant were allowed a cooling time of 30 minutes.

*H. P. Bennet, Kansas City (No. 3).* Dish used, flat-bottomed platinum. Desiccator, ordinary type Scheibler, 9" high and 13" in diameter. Fresh concentrated  $\text{H}_2\text{SO}_4$  used as desiccant. Number of dishes cooled in desiccator, two, for a period of 20 minutes. No difficulty was encountered.

*Daniel Banes, Chicago (No. 2).* Dishes of Pt, with Pt covers for each, cooled for 1 hour after removal from muffle, in individual aluminum desiccators, and magnesium perchlorate used as desiccant. All ashing in automatically-controlled muffle at  $550^\circ\text{C}$ . On closing muffle after fuming samples of marjoram, a rise in temperature over  $550^\circ$  (ca.  $580^\circ$ ) was noted. Muffle coils were slightly red. In subsequent work sample was fumed and set aside until muffle control registered  $550^\circ$ .

*David W. Williams, San Francisco (No. 9).* In the determination of ash on yellow mustard, it was noted that leaching and filtering of soluble matter after a preliminary burning off did not appear to expedite ashing. In fact an ashing without leaching was completed much more rapidly when the nitric acid treatment with

entire ash was used. (The purpose of the leaching was discussed previously by the Referee. The effect of direct treatment with  $\text{HNO}_3$  acid without leaching is shown by Williams, who obtained 0.12% lower ash by direct treatment than by the specified method.)

#### ASH IN PREPARED MUSTARD

The Association recommended study of the determination of ash in prepared mustard. Sufficient work was done to demonstrate that the official method is not satisfactory. Because of the presence of salt the method used for mustard flour may not be applicable. It is proposed to study this problem next year.

#### MOISTURE

Work on a direct determination of moisture to supplant the unsatisfactory indirect method now official consisted of a study of direct distillation methods and an attempt to apply a titration method after extraction of water with a suitable solvent.

Some of the methods recently proposed for direct determination of water (4) involve determination of increase of temperature necessary to cause complete miscibility of partly miscible organic liquids containing varying proportions of water. Others (5) depend upon titration with water of a mixture of ethyl alcohol and an organic liquid containing varying quantities of water, until cloudiness marks the end point. Smith and Bryant (6) determined water in organic liquids by titrating the acid liberated by hydrolysis of acetyl chloride by the water, in the presence of pyridine. The most interesting method for titrimetric determination of water is that proposed by Karl Fischer (7), who titrates the wet material with a solution of iodine, sulfur dioxide, and pyridine in methanol. The reagent, which serves as its own indicator, is extremely sensitive. The method has been used for the determination of water in cellulose by Mitchell (8), in oils and fats by Kaufmann and Funke (9) and Erlandsen (10), and in flour, margarine, cocoa powder, marmalade, and malt extract by Richter (11).

The Referee attempted to apply this method to spices. It appears to be necessary to extract the moisture with a suitable solvent before titration. It must also be determined that the reagent will not react with any of the many organic compounds present in spices, particularly in the oil. Although it is claimed to be specific for water, Smith, Bryant, and Mitchell (12) find aldehydes and ketones may interfere by formation of acetals and ketals with the large excess of methanol in the reagent, and the attendant liberation of water. This may be avoided by reducing the methanol content of the reagent.

Preliminary experiments with black pepper and cinnamon yielded low results, caused by incomplete extraction of water from the spice by methyl alcohol. The prospect of a rapid direct method and of the use of a small sample is so inviting that this study should be continued.

Many years ago this Association studied the direct distillation of water from spices by the Brown-Duval procedure (2), but the results were not satisfactory. Since then the Bidwell-Sterling, the Dean and Stark, and a large number of other tubes of various designs have attained wide use in the distillation of moisture from many food and industrial products. In the past few years a number of writers (13, 14, 15, 16, 17) have proposed new apparatus to overcome difficulties experienced with the Bidwell-Sterling apparatus, which is probably the most widely used in food analysis. The most suitable of these appeared to be that of de Loureiro (13). He pointed out that the chief demerit of the Bidwell-Sterling apparatus is the difficulty in preventing droplets of water from sticking to the walls of the condenser and collecting tube. He ascribes this difficulty to two causes: first, in an ordinary reflux condenser, toluene vapor being less volatile than water vapor, condenses below the water and so is unable to sweep down the condensed droplets of water; second, at neutral or acid reaction water has a greater affinity for glass than has toluene, and hence tends to adhere stubbornly to the walls of the apparatus. He proposed to correct these faults by changing the direction of flow of vapors and by keeping the surface of the glass alkaline.

An apparatus similar to that of de Loureiro was prepared by J. H. Cannon of this laboratory. When the apparatus was tested with water, complete recovery was obtained in 32 minutes, with two suckbacks, and the operation was satisfactory. However, when a sample of black pepper was distilled with toluene, the performance was unsatisfactory. After distillation for 2½ hours, during which time frequent attention was required and it was necessary to interrupt the distillation eight times to permit the toluene to suck back, only 92 per cent of the moisture had come over and the toluene in the condenser was still cloudy. After several similar experiences with cinnamon and marjoram, use of this apparatus was abandoned.

The use of an alkaline rinse on the walls of the condenser and tube, and of dipotassium phosphate in the distilling flask to neutralize volatile fatty acid as suggested by de Loureiro, was combined with the regular Bidwell-Sterling procedure. The alkaline salt was found to be of no advantage for the spices tested, but the alkaline rinse seemed to be of definite value.

The method submitted to the collaborators with four different spices provided for an alkaline rinse of the regular Bidwell-Sterling apparatus. The results are given in Table 9. The method follows:

#### MOISTURE

Clean the distilling tube receiver and condenser described in *Methods of Analysis*, A.O.A.C., 1940, 353, 3, with  $\text{Cr}_2\text{O}_3\text{-H}_2\text{SO}_4$  mixture, rinse thoroughly with water, then with approximately 0.5 N alcoholic KOH solution, and allow apparatus to drain for 10 minutes. Remove connecting stopper from condenser before cleaning, so that it remains dry. Place 40 grams of spice in the distilling flask and determine moisture as directed in 4.

The collaborators were requested to report whether it was necessary to use a tube brush or wire to dislodge water droplets.

TABLE 9.—*Collaborative results (%) on moisture in spices by toluene distillation*

ANALYST	BLACK PEPPER	MARJORAM	CINNAMON	SAGE
1	9.80	8.40	9.30	8.25
	9.85	8.48	9.45	8.18
	9.90			
2	9.32*	8.06*	9.11*	7.75*
	9.12*	7.83*	8.98*	7.93*
3	9.83	8.40	9.45	8.25
	9.80	8.45	9.38	
4	9.80	8.31	9.28	8.21
5	9.92	8.60	9.57	8.31
7	9.80	8.30	9.10	8.20
	9.63	8.18	9.08	8.13
9	9.75	8.39	(9.52)†	(8.50)†
Average	9.81	8.40	9.33	8.22
Maximum	9.92	8.60	9.57	8.31
Minimum	9.12	7.83	8.98	7.75
Standard deviation	0.08	0.12	0.16	0.05

\* Not included in standard deviation or average.

† Sample charred. Not used in calculations

#### COMMENTS OF COLLABORATORS

*S. D. Fine (No. 4).*—Tube brush not used. Time from 1 hour 10 minutes to 1 hour 25 minutes.

*F. M. Garfield (No. 5).*—Relative humidity of atmosphere was 75–85%. Time of distillation varied from 1 hour 30 minutes to 1 hour 45 minutes.

*F. J. McNall (No. 7).*—No necessity was found for using tube brush on condenser for Bidwell-Sterling tubes.

*Daniel Banas (No. 2).*—Tube brush unnecessary. However, pepper and marjoram distillations gave an uneven meniscus, and droplets of water collected on the sides of the Bidwell-Sterling tube. Treatment with Cu wire as suggested in *Methods of Analysis. A.O.A.C.*, 1940, p. 353, sufficed.

*D. W. Williams (No. 9).*—Moisture determinations were made according to instructions, but it was noticed that a localized charring and consequent destructive distillation on the sides of the flask occurred toward the end of the distillation. To avoid this an asbestos pad with a hole smaller than the periphery of the flask was placed on the asbestos gauze to prevent localized overheating. Only two determinations could be made with this device, since samples had been depleted.

Although in most cases it did not appear to be necessary to use a tube brush to push down last traces of water in the condenser, a rubber band attached to a copper

wire was used as a precaution. In a determination using water in toluene, an appreciable amount of water did adhere to the condenser and was dislodged by the rubber band.

In view of Williams' comments, it is advisable to specify that the flask be heated in an oil bath, or on a hot plate with an asbestos guard to prevent charring.

With the exception of one set of results, the agreement is fairly good for a distillation method.

In addition to the collaborators mentioned, the following men, all members of the Food and Drug Administration, took part in this work: A. E. Plumb, N. L. Knight, and the Referee.

#### SOIL CONTAMINANTS

The Association directed that studies be initiated on methods, other than ashing, for measuring soil contamination. The Referee made some preliminary tests on flotation procedures, but they were not entirely successful.

Soil contaminants may be of mineral, vegetable, or animal origin. The first classification would include small stones, clay, sand, metal, or earth containing more or less vegetable matter. Under the second classification would be more or less decayed leaves, stems, roots, stalks, etc. Fecal matter from animals would constitute the third class. Experiments with dried manure from cows, sheep, and horses indicated that these could not be separated from ground spices by differential flotation. As was expected, it was found that vegetable matter in the form of ground dried leaves and stems could not be separated from ground spices in this manner.

The method must be used chiefly, then, for clay, sand, stones, and loam. The Referee used as the test contaminant a clayey garden soil. The soil was dried and ground finely in a mortar. The ash content was 92.84 per cent, and the acid-insoluble ash 88.89 per cent. When a sample of this soil was floated in a separatory funnel with carbon tetrachloride for 20 minutes and the settlings were drawn off, dried, cooled, and weighed, the recovery was 93.8 per cent. When the recovered material was ignited, 88.8 per cent of the original sample remained.

Samples of black pepper and cinnamon, some containing added soil, were extracted with petroleum benzin to remove oil, and then floated in several solvents and mixtures. Carbon tetrachloride, chloroform, and petroleum benzin were used. The most successful procedure was to use chloroform with a small proportion of petroleum benzin for the first flotation, then to float the residue in carbon tetrachloride. In all cases, however, it was found that some of the soil was entrapped by the spice, and some of the spice was carried down with the soil.

For comparison, acid-insoluble ash was determined on the same samples. The results were more constant than those obtained by flotation, and gave better recoveries of added soil for this one sample of soil.

A flotation method appears to be of advantage only when the soil contaminant is acid-soluble, as limestone. It is doubtful whether such contamination is frequent. Under the circumstances, the Referee is inclined to doubt the usefulness of further work on flotation methods for soil contamination.

#### VOLATILE OIL IN SPICES

The associate referee did not submit a report on volatile oil this year.

#### STARCH AND SALT IN MUSTARD

Associate Referee Field studied various methods for determination of starch in mustard flour. The most promising one was subjected to collaborative study. Some of the results were excellent, but others were discordant. The associate referee believes that some minor modifications will correct the faults in the method. He recommends further collaborative study. The Referee concurs.

The associate referee also studied two methods for determination of salt in mustard flour. Both gave excellent results. These methods were not tested on prepared mustard, to which they are meant to apply. The Referee therefore disapproves of the associate referee's recommendation that one of these methods be adopted as tentative, and suggests that the method first be studied on prepared mustard. There is reason for suspecting that the official method for salt in prepared mustard, *Methods of Analysis, A.O.A.C.*, 1940, 474, 36, yields low results. It is recommended that sufficient work be done to determine whether this method should be dropped.

No work was done on volatile oil in mustard seed and other mustard products.

#### VINEGAR

Associate Referee Henry submitted reports by himself and also by Gulick and Sanders describing the extensive investigations carried out by them on phosphoric acid and caramel in vinegar.

Based on Gulick's findings, the associate referee recommends that the method described by Gulick be adopted as tentative, and studied collaboratively next year. The Referee, while recognizing the thorough study given this method by Gulick, and believing it will prove to be a satisfactory procedure does not favor its adoption as tentative until it has been subjected to collaborative study.

The associate referee submitted collaborative results on total, soluble, and insoluble  $P_2O_5$  obtained after ashing, and on total  $P_2O_5$  after wet digestion, the final determination being by the colorimetric molybdenum blue method used for fruit products. The agreement is fairly good for total  $P_2O_5$ , being slightly better for the wet digestion, on the whole, than for ashing. The variation is again considerable in the soluble and insoluble  $P_2O_5$  ratios on apple cider vinegar, and much greater for malt vinegar.

After all the work that has been done on this problem, it is still true that, as the associate referee says, "detection of less than 25 per cent adulterants [by means of ratio of soluble to insoluble  $P_2O_5$ ] in malt or apple cider vinegar would be difficult."

With these limitations in mind, the Referee believes that the methods should still be retained, with the modifications suggested by the associate referee, and, reluctantly, that further work on soluble and insoluble  $P_2O_5$  would be fruitless.

It has been noted by at least three workers in the Food and Drug Administration (18) that the official volumetric method for  $P_2O_5$  in fruits gives results about 4 per cent too high. Henry's results on malt vinegar by the two methods tend to bear out these findings. Presumably his directions to standardize the titrating solutions against "a sample of known phosphate content" would obviate the high results by the volumetric method.

The Referee does not approve of the directions suggested, to "express result as mg.  $P_2O_5$  per 100 ml. of vinegar," because he does not regard them as a legitimate part of a method of analysis.

The Association has no method for total  $P_2O_5$ , independent of the soluble and insoluble fractions. The associate referee recommends the insertion of such a method, with alternative procedures for preparing the solution, and two methods for final determination. One of these methods (wet digestion with subsequent colorimetric determination) is the method tested collaboratively by the Association on fruits and fruit products. The additional collaborative work performed this year makes the method worthy of adoption as official (first action). The other method is essentially that now official for vinegar, with the omission of the separation of soluble and insoluble  $P_2O_5$ . It is also considered to have been tested sufficiently to warrant adoption as official (first action).

The associate referee has made some progress in his studies on the "oxygen value" of vinegar, and proposes to continue these studies.

There have been recent publications by Edwards and Nanji (19) and Illing and Whittle (20) on the "ester value" of vinegars as a means of distinguishing between vinegar and commercial acetic acid. The Referee had occasion to use this value in deciding whether alcohol denatured with ethyl acetate had been used in making distilled vinegar. It is recommended that the Associate Referee on Vinegar investigate the usefulness of the "ester value" for vinegar.

#### SALAD DRESSINGS

Associate Referee Ryan conducted collaborative studies on salad dressings and submitted a number of recommendations. The Referee disapproves of the recommendation that the methods for preparation of sample and for determination of fat, with certain changes, be adopted as official (first action). The Referee approves of the recommended changes,

but recommends that the methods be continued as tentative until further collaborative work is done.

The Referee approves the recommendations for adoption of methods for sugar and for identification of oil as official (first action), and the recommendation that methods for determination of starch in salad dressings be studied.

#### RECOMMENDATIONS\*

It is recommended—

(1) That studies on the determination of total solids in vinegar be continued, with special reference to vinegars high in solids.

(2) That the official methods for determination of soluble and insoluble phosphoric acid in vinegar be amended in accordance with the recommendations of the associate referee except that reference to method of expressing results be omitted; and that no further work be done on these methods.

(3) That the methods for total phosphoric acid in vinegar described by the associate referee, with omission of the method for expressing results, be made official (first action).

(4) That the method for detection of caramel in vinegar described in the associate referee's report be studied collaboratively.

(5) That studies on the "oxygen value" of vinegar, including collaborative work, be continued.

(6) That studies on the "ester value" of vinegar be initiated.

(7) That the tentative method for determination of volatile oil in spices be further studied for spices other than marjoram and sage, with a view to adoption as official.

(8) That studies of methods for the determination of starch in mustard flour and prepared mustard be continued.

(9) That the official method for volatile oil in mustard seed be studied as to its application to different types and to other mustard products.

(10) That the method for determination of moisture in spices by toluene distillation described in this report be adopted as tentative, and studied further with a view to adoption as official.

(11) That studies on direct titration methods for determination of moisture in spices be continued.

(12) That the official method for ash in spices be dropped.

(13) That the method for ash in spices described in this report be adopted as tentative, and be further studied with a view to adoption as official.

(14) That the determination of ash in prepared mustard be studied.

(15) That studies on methods, other than ashing, for measuring soil contamination be discontinued.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 60 (1941).



(16) That the official (first action) method for the iodine number of paprika oil be made official (final action).

(17) That studies of methods for determination of salt in prepared mustard be continued.

(18) That the tentative method for total fat in mayonnaise and salad dressing be modified to provide for a 1 gram sample, and be subjected to further study.

(19) That the tentative methods for sugar, and identification of oil in mayonnaise and salad dressing be made official (first action).

(20) That the method for preparation of sample be modified according to the associate referee's suggestion, and be further studied.

(21) That studies be initiated on determination of starch in salad dressings.

#### REFERENCES CITED

- (1) BRINTON, *This Journal*, **2**, 201 (1917).
- (2) SINDALL, *Ibid.*, **2**, 197 (1917).
- (3) WICHMANN, *Ibid.*, **23**, 680 (1940).
- (4) BLADE, *Ind. Eng. Chem., Anal. Ed.*, **12**, 330 (1940).
- (5) BOTSET, *Ibid.*, **10**, 517 (1938).
- (6) SMITH and BRYANT, *J. Am. Chem. Soc.*, **57**, 841 (1935).
- (7) FISCHER, *Angew. Chem.*, **48**, 394 (1935).
- (8) MITCHELL, *Ind. Eng. Chem., Anal. Ed.*, **12**, 390 (1940).
- (9) KAUFMANN and FUNKE, *Fette Seifen*, **44**, 345 (1937).
- (10) ERLANDSEN, *Dairy Ind.*, **3**, 178 (1938).
- (11) RICHTER, *Angew. Chem.*, **48**, 776 (1935).
- (12) SMITH, BRYANT, and MITCHELL, *J. Am. Chem. Soc.*, **61**, 2407 (1939).
- (13) DE LOUREIRO, *This Journal*, **21**, 645 (1938).
- (14) CALDERWOOD and PIECHOWSKI, *Ind. Eng. Chem., Anal. Ed.*, **9**, 520 (1937).
- (15) LANGELAND and PRATT, *Ibid.*, **10**, 400 (1938).
- (16) BECKEL, SHARP, and MILNER, *Ibid.*, **11**, 425 (1939).
- (17) BAILEY, *Ibid.*, **9**, 568 (1937).
- (18) GERRITZ, private communication; FIELD and ALFEND, unpublished report, 1939.
- (19) EDWARDS and NANJI, *Analyst*, **63**, 410 (1938).
- (20) ILLING and WHITTLE, *Ibid.*, **64**, 329 (1939).

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No report on volatile constituents of spices was given by the associate referee. The paper presented, entitled "Volatile Oil in Cassia Bark," was published in *This Journal*, **24**, 461 (1941).

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#### REPORT ON VINEGARS

By ARTHUR M. HENRY (U. S. Food and Drug Administration,  
Atlanta, Ga.), Associate Referee

*Total solids in vinegar.*—Investigational work on the determination of total solids in vinegars, with special reference to vinegars high in solids, such as malt vinegar, was continued. No formal report is being submitted

at this time. No modification has been tried that will give results that can be duplicated at different times by this analysis with any greater accuracy than will the present official method. A few modifications have been suggested, but they have not been tried. It is believed that some of these are worthy of further work.

*Phosphoric acid.*—In connection with John W. Sanders, Jr., the Associate Referee did considerable work on the determination of total soluble and insoluble phosphoric acid in different types of vinegars. Collaborative work was also done. In addition to the present methods for soluble and insoluble phosphoric acid, the colorimetric method is being recommended for adoption. A method for total phosphoric acid is also being proposed, as the work has shown that in certain types of vinegars a determination of the total phosphoric acid is sufficient.

*Caramel in vinegar.*—H. Marion Gulick worked on this recommendation. He modified the Lichthardt method by the addition of formaldehyde to the original reagent. This modification seems to be more sensitive and gives better results than does the original reagent.

*Oxygen value.*—Some work was done on this recommendation. The method as outlined in the literature<sup>1</sup> gave a very poor end point to the titration. The work has largely been on improvement in the method. A modification of the published methods that has been worked out seems to give a sharp end point. It is planned to continue the investigation on authentic distilled vinegars and on solutions of glacial acetic acid. If this method gives promising results on distilled and acetic acid solutions, it will be submitted to collaborative study.

#### RECOMMENDATIONS\*

It is recommended—

(1) That studies on the determination of total solids in vinegars, with special reference to vinegars high in solids, be continued.

(2) That the revised method for soluble and insoluble phosphoric acid and the new method for total phosphoric acid submitted by Sanders and Henry (p. 684) be adopted as official (first action).

(3) That the method proposed by Gulick for the determination of caramel in vinegar (p. 691) be adopted as tentative, that it be submitted to collaborative study with a view to adoption as official, and that investigational studies of cider vinegars be continued to determine whether aging causes substances to form in cider vinegar which would give a precipitate with this reagent.

(4) That studies on the oxygen value of vinegar be continued to determine its usefulness in differentiating between distilled vinegars and solutions of commercial acetic acid.

<sup>1</sup> Edwards and Nanji, *Analyst*, 63, 410 (1938); H. J. Fisher, Connecticut Agr. Exp. Sta. Bull. 426, 21 (1938).

\* For report of Subcommittee C and action by this Association, see *This Journal*, 24, 60 (1941).

## REPORT ON PHOSPHORIC ACID IN VINEGAR

By JOHN W. SANDERS, JR., and ARTHUR M. HENRY (U. S. Food and Drug Administration, Atlanta, Ga.)

As a result of a thorough study of two samples of vinegar, Clarke, *This Journal*, 9, 440 (1926), cited the erratic results obtained for water-soluble and water-insoluble  $P_2O_5$  and suggested that total  $P_2O_5$  determination may have to be substituted. Further collaborative study by Clarke, *Ibid.*, 10, 490 (1927), showed that increasing the ashing temperature increased the water-soluble  $P_2O_5$ , but did not affect the total  $P_2O_5$ . Clarke and Feldbaum, *Ibid.*, 11, 499 (1928), confirmed the results of Clarke as given above and suggests the determination of total  $P_2O_5$  only. Henry, *Ibid.*, 16, 536 (1933), showed that vinegar could be ashed at 500°–550° C. without altering the soluble-insoluble ratio. Ashing below this temperature is impractical and above this it gives unreliable results. Shuman, *Ibid.*, 20, 402 (1937), confirms the work of the above collaborators in his study of cider vinegar. However, he shows that a considerable portion of  $P_2O_5$  is actually lost in the ashing of malt vinegar and further emphasizes the unreliability of water-soluble and water-insoluble  $P_2O_5$  determination.

The writers continued this study of the water-soluble and water-insoluble  $P_2O_5$  in apple cider, distilled, malt, molasses, and corn sugar vinegar. It should be noted, however, that practically all the work cited above was with cider vinegar. Shuman, *Ibid.*, did some work with malt vinegar, but the data obtained do not warrant any definite conclusions.

In view of the fact that the ratio of water-soluble to water-insoluble  $P_2O_5$  is of importance in distinguishing between different types of vinegar and in detecting adulterants in certain types of vinegar, a total disregard of this determination seems unwarranted unless it can be shown that such values are too unreliable for practical application.

The method outlined below was adopted in order to show the effect of length of time and of temperature of ashing on the ratio of water-soluble to water-insoluble  $P_2O_5$  in different types of vinegar. In addition, the Zinzadze colorimetric method, as modified by Gerritz and others, was adapted to this determination. Gerritz has shown this method to be reliable for the determination of small quantities of  $P_2O_5$  in fruit and fruit products.

## PROCEDURE

Pipet into a platinum dish an aliquot of vinegar representing 1–4 mg. of  $P_2O_5$  and evaporate to dryness on the steam bath. Heat in an electric muffle at the designated temperature (controlled automatically). In order to eliminate temperature fluctuations as much as possible, cover the bottom of the muffle with an asbestos mat approximately one-fourth inch thick. (However, the maximum fluctuation in any part of the muffle may be regarded as less than  $\pm 25^\circ$  C.) After ashing for the length of time and at the temperature desired, remove the sample from the muffle, and allow to cool. Warm on the steam bath with 25 ml. of distilled water and filter

through a quantitative filter paper into a 100 ml. volumetric flask. Wash thoroughly with hot water. Cool, dilute to mark, and mix thoroughly (water-soluble  $P_2O_5$ ).

Replace the filter paper in the platinum dish and again ignite in the muffle. Remove, cool, add 25 ml. of  $H_2SO_4$  (1+4), and warm on the steam bath. Remove, and filter, receiving the filtrate in a 100 ml. volumetric flask. Wash thoroughly with hot water. Cool, dilute to mark, and mix thoroughly (water-insoluble  $P_2O_5$ ).

Determine the quantity of  $P_2O_5$  in both solutions (using 20 ml. aliquot) by the colorimetric method proposed by Gerritz for  $P_2O_5$  in fruit and fruit products (private communication)

TABLE 1

TEMPERATURE	TIME	SOLUBLE $P_2O_5$	INSOLUBLE $P_2O_5$	TOTAL $P_2O_5$
° C.	hours	mg./100 ml.	mg./100 ml.	mg./100 ml.
<i>Sample F, malt vinegar (colorimetric method)</i>				
425	2	14.5	55.5	70.0
		18.0	55.0	73.0
	4	26.5	46.5	73.0
		26.5	48.0	74.5
	6	27.0	44.0	71.0
525	2	27.5	49.0	76.5
		53.0	25.0	78.0
	4	49.0	28.5	77.5
		56.5	19.5	76.0
	6	52.5	22.5	75.0
550	2	55.0	21.0	76.0
		57.0	18.0	75.0
	4	40.5	32.5	73.0
		36.5	36.0	72.5
	6	43.0	28.5	71.5
625	2	31.0	43.5	74.5
		43.0	30.5	73.5
	4	42.5	31.0	73.5
		32.0	42.0	74.0
	6	29.5	44.0	73.5
725	2	26.5	48.0	74.5
		26.0	49.0	75.0
	4	22.5	53.0	75.5
		20.5	53.5	74.0
	6	48.0	23.0	71.0
825	2	46.5	25.0	71.5
		45.5	25.5	71.0
	4	44.5	27.5	72.0
		45.0	25.0	70.0
	6	46.5	23.0	69.5
	1	29.0	44.0	73.0
		26.5	47.5	74.0
	3	23.0	49.0	72.0
		24.5	48.0	72.5
	5	25.0	49.0	74.0
		24.0	49.0	73.0

TABLE 1.—Continued

TEMPERATURE	TIME	SOLUBLE $P_2O_5$	INSOLUBLE $P_2O_5$	TOTAL $P_2O_5$
°C.	hours	mg./100 ml.	mg./100 ml.	mg./100 ml.
<i>Sample F, malt vinegar (volumetric method)</i>				
425	2	21.6	56.1	77.7
		25.1	52.3	77.4
525	2	48.7	28.2	76.9
		46.3	30.2	76.5
625	2	49.3	26.9	76.2
		46.8	29.8	76.6
725	2	40.5	36.1	76.6
		41.0	35.8	76.8
<i>Sample A, apple cider vinegar (colorimetric method)</i>				
525	5	7.50	6.30	13.80
		7.50	6.30	13.80
625	6	6.70	6.65	13.35
		6.65	6.70	13.35
	6	8.25	5.25	13.50
		7.88	5.43	13.31
725	6	8.25	5.20	13.45
		7.60	5.65	13.25
		8.25	5.25	13.50
		8.50	4.85	13.25
<i>Sample B, distilled vinegar (colorimetric method)</i>				
525	3	0.73	1.46	2.19
		0.63	1.58	2.21
625	5	0.61	—	—
		0.43	1.67	2.10
	6	0.41	1.72	2.13
		0.54	1.57	2.11
725	9	0.20	1.89	2.09
		0.18	1.90	2.08
		0.13	1.95	2.08
725	9	0.17	1.90	2.07
		0.17	1.91	2.08
		0.16	1.91	2.07
<i>Sample D, molasses vinegar (colorimetric method)</i>				
525	2	Trace	5.60	5.7
525	2	Trace	5.76	5.8
525	5	Trace	5.92	6.0
525	5	Trace	5.80	5.9
<i>Sample E, corn sugar vinegar (colorimetric method)</i>				
525	2	Trace	6.32	6.4
525	2	Trace	6.24	6.3
525	5	Trace	6.16	6.2
525	5	Trace	6.16	6.2

In general, the results (Table 1) are erratic, even with duplicate samples that were ashed at the same time and adjacent to each other in the muffle. At temperatures of 525° C. and above, the ash was white and fluffy in all cases. Perhaps if larger aliquots had been used, ashing would have been more difficult and a longer time required.

However, the character of the ash obtained and the high value of the water-insoluble  $P_2O_5$  show that an ashing temperature of 425° C. is too low. Apparently,  $P_2O_5$  that should have been soluble is shown as water-insoluble because of incomplete destruction of organic matter and occlusion of the  $P_2O_5$  by the charred mass.

No explanation is offered for the high value of the water-soluble  $P_2O_5$  obtained at 725° C. However, the  $P_2O_5$  is also much lower, which indicates a loss during ashing. The results for total  $P_2O_5$  indicate a loss with increase of the temperature of ashing.

The results obtained by the volumetric method for water-soluble and water-insoluble  $P_2O_5$  show poor agreement, but those for total  $P_2O_5$  are in much closer agreement. However, the values for the total  $P_2O_5$  are somewhat higher than those obtained by the colorimetric method.

The results for Sample A, apple cider vinegar, indicate that increasing the temperature of ashing increases the quantity of water-soluble  $P_2O_5$ . The higher value of water-soluble  $P_2O_5$  content with 5 hours' ashing is probably due to incomplete destruction of organic matter as shown by the greyish color. There is also a slight decrease in total  $P_2O_5$  with increase of ashing temperature, but the apparent loss is almost negligible.

The results for Sample B, distilled vinegar, indicate that the temperature and length of time of ashing affect the ratio of water-soluble to water-insoluble  $P_2O_5$  in distilled vinegar. There is a decrease of water-soluble and total  $P_2O_5$ , but an increase of water-insoluble  $P_2O_5$  with increase of length of time or temperature of ashing. However, the small number of analyses do not warrant such a conclusive statement.

The results shown for molasses and corn sugar vinegar indicate that practically all the  $P_2O_5$  in the ash is insoluble regardless of the length of time of ashing. Experiments were not conducted to determine the effect of the temperature of ashing. The water-soluble  $P_2O_5$  in both molasses and corn sugar vinegar represents approximately 0.05–0.1 mg./100 ml. However, only 25 ml. of vinegar was represented in the color development flask, and it was difficult to obtain an accurate reading. It is from this approximation that the values for the total  $P_2O_5$  are obtained.

These results confirm those of previous investigation (*loc. cit.*) that the temperature of ashing affects the ratio of the water-soluble to water-insoluble  $P_2O_5$  in vinegar. However, the effect is more pronounced in malt than in apple cider or distilled vinegar. Furthermore, the results indicate that even the length of time of ashing alters this ratio to a lesser degree.

TABLE 2.—Collaborative results

ANALYST	SAMPLE A—APPLE CIDER					SAMPLE B—DISTILLED					SAMPLE C—MALT					SAMPLE D—MOLASSES					SAMPLE E—CORN SUGAR				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
W. H. Naylor Seattle	60	6.08	7.88	13.96	13.9	60	0.104	2.13	2.23	2.31	60	25.6	66.0	91.6	96.0	60	0.048	6.28	6.33	6.2	60	0.020	6.32	6.34	6.3
	180	6.48	7.20	13.68	—	180	0.148	2.12	2.27	—	180	24.0	73.2	97.2	—	180	0.048	6.24	6.29	—	180	0.028	6.60	6.63	—
L. W. Ferris Buffalo	80	5.08	7.76	12.84	13.25	80	0.140	1.95	2.09	2.11	80	20.0	71.2	91.2	94.8	80	0.084	6.04	6.12	6.10	80	0	6.40	6.40	6.28
	80	6.60	6.76	13.36	13.10	80	0.236	1.86	2.10	2.14	80	24.0	70.0	94.0	94.5	80	0.140	6.92	6.06	6.15	80	0.120	6.08	6.30	6.28
J. H. Loughrey Boston	105	5.60	7.50	13.10	13.67	70	0.17	2.73	2.90	2.13	70	18.2	63.0	81.2	93.75	80	0.16	6.0	6.2	5.95	80	0.10	6.20	6.30	—
	105	5.88	7.64	13.52	13.17	70	0.16	2.80	2.96	2.10	70	18.4	66.0	84.4	95.00	80	0.17	6.0	6.2	5.30*	80	0.10	6.00	6.10	6.35
D. A. Holday San Francisco	90	7.32	7.00	14.32	13.55	90	0.06	1.95	2.01	2.08	90	20.60	76.80	97.40	97.0	60	0.06	6.00	6.06	6.33	60	0.06	6.20	6.26	6.63
	90	6.52	6.60	13.12	13.50	90	0.08	2.00	2.08	2.08	90	19.60	76.80	96.40	96.0	60	0.07	6.08	6.15	6.28	60	0.07	6.28	6.35	6.55
S. D. Fine St. Louis	115	5.3	7.8	13.1	13.4	80	0.2	1.9	2.1	2.1	80	27.8	61.6	89.4	93.3	80	0.1	5.8	5.9	6.1	80	0.06	6.0	6.1	6.3
	115	5.4	7.5	12.9	13.4	80	0.2	1.9	2.1	2.1	80	27.4	61.0	88.4	93.5	80	0.1	6.0	6.1	6.1	80	0.06	6.0	6.1	6.3
G. Q. Lipecomb Baltimore	80	5.28	7.60	12.88	13.00	80	0.132	1.96	2.09	2.00	80	20.8	75.4	96.2	99.25	80	0.120	—	—	9.40*	80	0.148	7.04	7.19*	7.40
	80	5.28	7.60	12.88	13.00	80	0.132	1.96	2.09	2.10	80	20.8	74.8	95.6	97.50	80	0.120	9.20*	9.32*	9.50*	80	0.148	6.40	6.55	7.30
J. L. Hogan New York	80	8.00	5.40	13.40	—	80	0.61	1.37	1.98	—	80	40.40	50.00	90.40	—	80	0.29	5.92	6.21	—	80	0.31	6.08	6.39	—
	80	8.00	5.36	13.36	—	80	0.60	1.37	1.97	—	80	41.80	46.00	87.80	—	80	0.28	5.92	6.20	—	80	0.31	6.04	6.35	—
C. D. Schiffman Atlanta	110	6.80	7.76	14.56	13.70	110	0.44	1.90	2.34	2.21	110	28.0	69.2	97.2	98.0	110	0.24	6.40	6.64	6.64	110	0.12	6.56	6.68	6.64
	110	6.00	7.76	13.76	13.70	110	0.42	1.90	2.32	2.21	110	26.0	66.0	92.0	98.0	110	0.26	6.24	6.50	6.64	110	0.23	6.40	6.63	6.64
R. I. Martens Chicago	80	6.9	6.4	13.3	13.0	80	0.1	2.0	2.1	2.1	80	22.2	67.2	89.4	91.8	80	0.1	5.9	6.0	5.8	80	0.1	6.1	6.2	5.9
	80	6.6	6.6	13.2	—	80	0.2	2.0	2.2	—	80	24.4	65.6	90.0	98.3	80	0.1	6.2	6.3	—	80	0.1	6.3	6.4	5.9
	80	6.4	6.8	13.2	—	80	0.2	2.0	2.2	—	130	23.8	64.6	88.4	91.0	80	0.1	6.2	6.3	—	80	0.1	6.3	6.4	5.9
											130	21.4	67.6	89.0											
J. W. Sanders, Jr. Atlanta	80	6.47	7.03	13.50	13.33	80	0.49	1.62	2.11	2.10	80	44.9	48.5	93.4	93.3	80	trace	6.24	6.3	6.24	80	trace	6.48	6.5	6.24
	80	6.03	7.20	13.23	13.33	80	0.46	1.66	2.12	2.10	80	46.5	47.6	94.1	93.5	80	trace	6.08	6.1	6.16	80	trace	6.64	6.7	6.24
Harry Shuman Philadelphia	80	5.92	7.40	13.32	—	80	0.40	1.69	2.09	—	80	35.5	55.1	90.6	—	80	0.10	6.12	6.22	—	80	0.15	6.20	6.35	—
	80	5.96	7.72	13.68	—	80	0.49	1.63	2.12	—	80	29.6	60.0	89.6	—	80	0.10	6.16	6.26	—	80	0.08	6.32	6.40	—
Average																									
Minimum				13.4	13.4				2.2	2.1					91.4	94.7								6.4	6.3
Maximum				12.8	13.0				1.97	2.0					81.2	88.3								6.1	5.9
				14.56	13.9				2.96	2.31					97.4	99.25								6.68	6.64

\* Not included in average, minimum, or maximum.

## COLLABORATIVE STUDY

The collaborators were requested to ash the vinegar by the present A.O.A.C. method and to determine the water-soluble and water-insoluble  $P_2O_5$  by the Zinzadze colorimetric method (modified by Gerritz and others). However, since the presence of nitric acid renders this method useless or inaccurate, the water-insoluble ash must be taken up in sulfuric acid.

In addition, the collaborators were requested to determine the total  $P_2O_5$  by a wet digestion method similar to that proposed by Gerritz for fruit and fruit products. The results obtained by the collaborators are shown in Table 2. The vertical columns represent the following results: I, total ashing time before separation of water-soluble and water-insoluble ash in minutes; II, water-soluble  $P_2O_5$ , g./100 ml.; III, water-insoluble  $P_2O_5$ , g./100 ml.; IV, total  $P_2O_5$  by ashing method (II & III), g./100 ml.; and V, total  $P_2O_5$  by wet digestion method, g./100 ml.

## COMMENTS BY COLLABORATORS

Analyst Hogan of the New York Station had difficulty in filtering the malt vinegar in the separation of soluble and insoluble ash. He suggests the possibility of a straight ashing of perhaps 30 minutes, which seemed ample for vinegars other than apple cider. In view of early work this seemed advisable, but it is desirable to determine the water-soluble and water-insoluble  $P_2O_5$  from the ash determinations.

## DISCUSSION OF RESULTS

In general, the results reported by different analysts are in fair agreement for total  $P_2O_5$ , whether by the wet digestion method or the ashing method. It is noted, however, that the results for malt vinegar (Sample C) are somewhat lower by the latter method, which indicates a loss of  $P_2O_5$  on ashing. In general, this is not true of the other four types of vinegar.

Although the alkaline number of malt vinegar is lower than that of any of the others, the difference does not seem large enough to justify this factor as the logical reason. However, previous investigators have shown the alkaline number to be important in the ashing of materials for phosphate determinations.

In general, the variations between results reported by different analysts for water-soluble and water-insoluble  $P_2O_5$  are so great that detection of less than 25 per cent adulterants in malt or apple cider vinegar would be difficult. Even duplicate analyses by the same analyst are not in good agreement.

Since the most erratic results were obtained on water-soluble and water-insoluble  $P_2O_5$  in apple cider and malt vinegar, the following results obtained by varying the ashing time are given in Table 3 (outlined method with the variation given in table).



TABLE 3.—Results on apple cider and malt vinegars from varying periods of ashing

VARIATION	SOLUBLE $P_2O_5$	INSOLUBLE $P_2O_5$	TOTAL $P_2O_5$
	mg./100 ml.	mg./100 ml.	mg./100 ml.
<i>Sample A, apple cider vinegar</i>			
3rd ignition: 10 min.	6.53	6.53	13.06
3rd ignition: 1 hr.	6.87	6.23	13.14
3rd ignition: 1 hr.	6.67	6.40	13.07
<i>Sample C, malt vinegar</i>			
2nd ignition: 1 hr.	36.2	56.1	92.3
2nd ignition: 1 hr.	38.0	54.6	92.6
3rd ignition: 10 min.	30.4	61.2	91.6
3rd ignition: 1 hr.	30.8	61.2	92.0
3rd ignition: 1 hr.	29.2	63.2	92.4

These results (Table 3) do not indicate that the slight differences of ashing time caused the differences in the results shown in the other tables.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the methods for soluble and insoluble phosphoric acid, official, *Methods of Analysis, A.O.A.C.*, 1940, be changed to read as follows:

Page 478, 63: Proceed as directed under II, 9 or 12, or XXVI, 41, using solution obtained under 62. If either volumetric or colorimetric method is used, standardize with a sample of known phosphate content. Express results as mg. of  $P_2O_5$  per 100 ml. of vinegar.

64: Dissolve water-insoluble ash, 61, in approximately 50 ml. of boiling  $HNO_3$  (1+8). Use 25 ml. of  $H_2SO_4$  (1+9) if colorimetric method is used and proceed as directed under II, 9 or 12, or XXVI, 41. If either volumetric or colorimetric method is used, standardize with a sample of known phosphate content. Express result as mg. of  $P_2O_5$  per 100 ml. of vinegar.

(2) That a method for the determination of total phosphoric acid be included to read as follows:

Dissolve ash, 60, or both soluble and insoluble ash, 61, in approximately 50 ml. of boiling  $HNO_3$  (1+8). Use 25 ml. of  $H_2SO_4$  (1+9) if colorimetric method is used, and proceed as directed under II, 9 or 12, or XXVI, 41. If either volumetric or colorimetric method is used, standardize with a sample of known phosphate content. Express result as mg. of  $P_2O_5$  per 100 ml. of vinegar. If desired, digest the vinegar as directed in XXVI, 40, instead of using ash from 60.

The following additional references may also be consulted: *This Journal*, 21, 89 (1938); 22, 121, 131, 167 (1939).

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 60 (1941).

## REPORT ON DETECTION OF CARAMEL IN VINEGAR

By H. MARION GULICK (U. S. Food and Drug Administration,  
Atlanta, Ga.)

Lichthardt<sup>1</sup> describes a reagent consisting of a dilute solution of tannic and sulfuric acids, which was used to detect caramel in vanilla extracts and in liquors. Only a few tests were made to determine the presence of caramel in vinegars but good results were claimed.

Cook and Miller, *This Journal*, **22**, 588 (1939), made a series of tests, using the Lichthardt reagent, to detect caramel in vinegar. Their results indicate that some vinegars, particularly old ones, tend to give a precipitate when no caramel is present. Also some vinegars with added caramel did not give a definite precipitation with the reagent unless heating was very carefully controlled. The tests reported by Cook and Miller were made with added caramel present in a concentration of 1 part in 1000 in the sample tested. It is quite possible that samples would be encountered with as little as one-sixth of this amount of caramel present.

In a series of tests recently made, it was found that the Lichthardt reagent does not always give a positive test when caramel is present in small concentrations. It was learned, however, that if the Lichthardt reagent is modified by the addition of formaldehyde, its sensitivity and reliability are increased.

The Lichthardt reagent is prepared as follows as suggested by Cook and Miller:

Dissolve 9.8 grams of tannic acid in approximately 300 ml. of water, add 4 ml. of  $H_2SO_4$ , and dilute to 490 ml.

The reagent was modified by adding 4 ml. of formaldehyde solution to 96 ml. of prepared reagent. The reagent does not keep more than one day after the addition of formaldehyde.

Cook and Miller used the Lichthardt reagent by adding 5 ml. of sample to 5 ml. of reagent and heating at 40°–48° C. for 4 minutes. For the tests described here, the modified reagent was used by mixing 5 ml. of sample with 5 ml. of reagent in a test tube, which is immersed in a beaker of boiling water for 4 minutes. After the tube has been allowed to stand overnight, a brown precipitate, which settles to the bottom, is considered to be a positive test. In some instances, a blue-grey precipitate is observed, but this is to be ignored unless there is also a quantity of brown precipitate in the bottom of the tube.

Preliminary tests with the modified reagent showed that good results could be obtained by heating in boiling water, and as the sensitivity of the reagent was found to be satisfactory in all cases, this procedure is recommended as being more convenient than the controlled heating,

<sup>1</sup> *J. Ind. Eng. Chem.*, **2**, 389 (1910).

TABLE 1.—*Tests on vinegar with and without added caramel*

VINEGAR	TYPE	ADDED CARAMEL	REAGENT USED:	
			LICHTHARDT	MODIFIED
A	Cider	None	—	—
M	Cider	None	—	—
R	Cider	None	+	—*
F	White Distilled	None	—	—
B	Distilled-colored	None	—	+
L	Distilled-colored	None	+	+
N	Distilled-colored	None	+	+
Q	Distilled-colored	None	+	+
K	Distilled and Cider	None	+	+
P	Distilled and Cider	None	+	+
C	Malt	None	+	+
D	Molasses	None	+	+
E	Corn Sugar	None	—	+
A	Cider	G 1:1000	+	
A	Cider	G 1:2500		+
A	Cider	S 1:4000	+	+
A	Cider	T 1:4000	+	+
M	Cider	S 1:4000	+	+
M	Cider	T 1:4000	+	+
R	Cider	S 1:4000	+	+
R	Cider	T 1:4000	+	+
F	White Distilled	G 1:1000	+	
F	White Distilled	G 1:2500		+
F	White Distilled	S 1:4000	+	+
F	White Distilled	T 1:4000	+	+
B	Distilled-colored	G 1:1000	+	

\* Cases showing difference between the two reagents.

TABLE 2.—*Tests on solutions of caramel*

CARAMEL	DILUTION	REAGENT USED:	
		LICHTHARDT	MODIFIED
G	1:2000		+
G	1:5000		+
H	1:2000	+	+
H	1:4000	?	+
J	1:2000	+	+
J	1:4000	+	+
S	1:2000	+	+
S	1:4000	+	+
S	1:6000	+	+
T	1:2000	+	+
T	1:4000	+	+
T	1:6000	?	+

\* Cases showing difference between the two reagents.

which Cook and Miller found necessary when using the original Licht-hardt reagent.

The five samples of caramel available for test showed quite a range of viscosity and some showed a variation in coloring power. Sample G has less than half the coloring power of Samples H, J, or S. Sample T has nearly twice the coloring power of Samples H, J, or S. Samples H, J, and S have almost identical coloring power. Coloring power was estimated by comparing the color of solutions diluted 1:500 and 1:1000. The viscosity of Sample S is so great that it will just pour at room temperature and the viscosity of Sample J is almost as great. Sample G is quite fluid, and the viscosities of Samples H and T range between these extremes. If caramel H, J, or S is diluted 1 part in 4000 the depth of color is approximately the same as that of vinegar B. Dilution to 1 part in 6000 gives a color approximately equal to that of vinegar L, which contains declared caramel.

A series of tests was made to determine the effect of alcohol. Mixtures were prepared from caramel H in concentrations of 1:2000 and 1:4000 containing 5, 10, and 30 per cent of alcohol. In all cases a positive test was obtained when the modified reagent was used.

TABLE 3.—*Effect of added alcohol*

TESTS WITH MODIFIED REAGENT USING SOLUTIONS OF CARAMEL H:		
ALCOHOL PRESENT	CONCENTRATION OF CARAMEL	RESULT
<i>per cent</i>		
None	1:2000	Positive
5	1:2000	Positive
10	1:2000	Positive
30	1:2000	Positive
None	1:4000	Positive
5	1:4000	Positive
10	1:4000	Positive
30	1:4000	Positive

*Vinegar Samples Used*

- A White House Pure Apple Cider Vinegar, National Fruit Products Co., Winchester, Va. (Composite of 4 gallons.)
- B Monumental Distilled Vinegar—Colored with Caramel, National Fruit Products Co., Washington, D. C. (Composite of 4 gallons.)
- C Heinz Pure Malt Vinegar, H. J. Heinz Co., Pittsburgh, Pa. (Composite of 8 pints.)
- D Molasses Vinegar, Speas Manufacturing Co., Kansas City, Mo.
- E Corn Sugar Vinegar, Manufactured by Alton Vinegar Co.
- F White Distilled Vinegar, H. J. Heinz Co., Pittsburgh, Pa.
- K 70% Distilled 30% Apple Cider Vinegars—Speas Brand, Speas Co. General Offices, Kansas City, Mo. (Collected 4/7/39.)
- L Distilled Vinegar—Colored, Riverside Club, William Waddell Co., Atlanta, Ga. (Collected 4/12/39.)

- M Pure Apple Cider Vinegar, Apple Pie Ridge, Shenandoah Valley Apple Cider & Vinegar Co. (Collected Jan., 1939.)
- N Distilled Vinegar with Caramel, Georgia Belle, Bottled by Standard Vinegar Co., Atlanta, Ga. (Collected 5/31/40.)
- P 65% Distilled 35% Apple Cider Vinegar, Harvest Moon, Packed for Harvest Products Co., Atlanta, Ga. (Collected Jan., 1939.)
- Q Distilled Vinegar—Colored with Caramel, Georgia Boy, Packed by Georgia Boy Products Co., Savannah, Ga. (Collected 2/23/39.)
- R Apple Cider Vinegar, Wayne County Brand, Wayne County Produce Co., Green Point, L. I. (Collected 5/14/40.)

#### *Caramel Samples Used*

- G McCormick & Co., Baltimore, Md.
- H Penick & Ford, Inc., Cedar Rapids, Iowa. Labeled for Soda Fountain use.
- J Burghausen Chemical Co., Cincinnati, Ohio.
- S Fries & Fries, New York City.
- T Union Sales Corp., Columbus, Inc., Pennant Brand, Acid Proof.

### DISCUSSION OF RESULTS

In all cases, except for cider vinegar R, when direct comparison was made between the original and the modified reagents, the amount of precipitate obtained with the modified reagent was equal to, or greater than that obtained with the original reagent. Cider vinegar R gives a positive test with the original reagent but a negative test with the modified reagent. It is probable that this cider vinegar does not actually contain caramel.

Distilled vinegar B is labeled as containing added caramel although the color is rather light. It was not possible to obtain a positive test with this vinegar except by using the modified reagent. The concentration of caramel present is probably less than the sensitivity of the original reagent.

Vinegars K and P, blends of cider and distilled vinegar, do not declare caramel on the label but have more color than would be expected from the declared percentage of cider vinegar. The positive tests obtained with these vinegars no doubt indicate added caramel.

Malt, corn sugar, and molasses vinegars appear normally to contain caramel.

### CONCLUSIONS

Vinegar and caramel are both products of somewhat variable chemical constitution.

The caramel present in some samples of vinegar may be as little as 1 part in 4000 or 6000.

The addition of formaldehyde to the Lichthardt reagent increases its sensitivity to low concentrations of caramel, making it possible to detect its presence in concentrations as low as 1:400 or 1:6000. This increased sensitivity of the reagent reduces the likelihood of obtaining doubtful or borderline results.

Alcohol may be present in amounts up to 30 per cent without appreciable effect on the results obtained.

#### RECOMMENDATIONS\*

The following method is proposed as a test for caramel in vinegar:

*Reagent.*—Dissolve 9.8 grams of tannic acid in approximately 300 ml. of water, add 4 ml. of  $H_2SO_4$ , and dilute to 490 ml.

Prepare reagent for use by adding 4 ml. of formaldehyde solution to 96 ml. of the above stock solution. Prepare fresh reagent for each day's use. The stock solution will keep for a month.

*Determination.*—Mix 5 ml. of sample to be tested in a test tube with 5 ml. of the reagent and immerse for 4 minutes in boiling water. Allow to stand overnight. A brown precipitate settled to the bottom of the tube indicates a positive test for caramel. A grey precipitate should be disregarded.

It is recommended that collaborative work be done with a greater variety of vinegars and caramels from different sources. New cider vinegars should be submitted to periodical tests with this reagent for a year or so to determine whether aging would cause substances that would give a precipitate.

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#### REPORT ON SALAD DRESSINGS

By L. T. RYAN (State Laboratories Department,  
Bismarck, N. D.), *Associate Referee*

At the 1939 meeting of the Association it was recommended by the referee, *This Journal*, 23, 66 (1940), that the tentative methods for preparation of sample, for the determination of sugars and fat, and for composition and identification of oil in salad dressings be studied collaboratively. To carry out these recommendations the work recorded here was undertaken.

The last report on these products was made by Lepper and Vorhes, *Ibid.*, 16, 548 (1933), and it seems that their methods, with the modifications recommended by the referee, *Ibid.*, 23, 87 (1940), and approved by the Association, would merit adoption as official if as satisfactory results could be obtained as were recorded in this previous project.

#### METHODS

All references to procedure will be found in *Methods of Analysis*, A.O.A.C., 1940.

*Preparation of sample.*—Before removing any portion of sample for analysis, transfer to a suitable container, such as a glass fruit jar of larger capacity than the volume of the sample, and mix until homogeneous with a spatula (2-3 minutes should be sufficient). Repeat mixing before each subsequent portion is removed for analysis if sample has stood for any appreciable length of time. For the various determinations, take approximately the quantity directed and weigh. (A light 100

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 60 (1941)

ml. flask fitted with a straight glass tube and over-size rubber bulb makes a suitable weighing bottle.)

**Total solids.**—Weigh accurately by difference from a weighing bottle into an aluminum dish about 2 grams of sample and proceed as directed under XXXIII, 2 and 3.

**Reducing sugars before inversion.**—Weigh 20 grams of the sample into a wide-mouthed 4 oz. bottle and extract the oil by adding about 80 ml. of petroleum benzin, shaking, and centrifuging. Draw off as much as possible of the petroleum benzin solution (conveniently done by using suction and a short-stemmed pipet), and repeat the treatment with petroleum benzin until all the oil has been removed (indicated by absence of color in the solvent—usually about four extractions are required). Reserve the ether solution for identification of the oil. Remove the petroleum benzin from the residue with a current of air and transfer the residue with water to a 100 ml. volumetric flask. Add 5–10 ml. of a fresh solution of  $\text{HPO}_3$  (prepared by dissolving 5 grams of the transparent lumps or sticks in cold water and diluting to 100 ml.), mix thoroughly, dilute to volume, and filter. Transfer 80 ml. of the filtrate, or as large an aliquot as possible, to a 100 ml. flask; neutralize with  $\text{NaOH}$  (1+1), using phenolphthalein indicator; cool, dilute to the mark, and determine reducing sugars on an aliquot as directed under XXXIV, 38. Calculate to invert sugar.

Some dressings, particularly those containing starch, can not be clarified by the above method. In such instances, remove the oil as directed under XXII, 20, using 1 ml. of strong  $\text{NH}_4\text{OH}$  solution and 5 ml. of alcohol for every gram of sample. Transfer the residue to a 250 ml. flask with alcohol, 50% by volume, and proceed as directed under XXVII, 28, and XXXIV, 37 and 38.

**Reducing sugars after inversion.**—Invert an aliquot of the solution from above, as directed under XXXIV, 23(b) or (c), and determine the reducing sugars in the inverted solution as directed under XXXIV, 37 and 38. Calculate to invert sugar.

**Sucrose.**—Subtract the percentage of invert sugar obtained before inversion, from that obtained after inversion, and multiply the difference by 0.95.

**Total acidity.**—Proceed as directed under XXXIII, 49.

**Total nitrogen.**—Proceed as directed under XXXIII, 50.

**Total phosphoric acid ( $\text{P}_2\text{O}_5$ ).**—Proceed as directed under XXXIII, 51.

**Total fat.**—Using a 1 gram sample, proceed as directed under XXXIII, 52.

**Calculation of composition.**—Proceed as directed under XXXIII, 53.

**Identification of oil.**—Proceed as directed under XXXIII, 54.

#### MODIFICATIONS IN PROCEDURE

In the preparation of sample the addition of the directions to mix with a spatula in a glass jar was based on the findings of C. D. Schiffman (personal communication) of the Atlanta Station of the Food and Drug Administration, who found that mechanical stirrers did not give complete mixing, whereas by the spatula method mixing was completed in 2–3 minutes. The writer had also obtained satisfactory mixing by this method in the analysis of commercial dressings.

In the determination of total fat it was found by several analysts that good results could not be obtained with a 2 gram sample. Analyst Schiffman found that good duplicate determinations were obtained when he used a 1 gram sample, while the writer and a co-worker had found that this difficulty could be overcome by using more extractions than specified.

As it seemed to be more desirable to reduce the final fat to be weighed to less than one gram, the method was altered to allow for the smaller sample.

#### PREPARATION OF COLLABORATIVE SAMPLES

Two samples were prepared for the collaborative work, a normal mayonnaise and a dressing similar in formula to the salad dressings found on the present-day market.

The materials used, with the exception of sugar and starch, and the analyses were essentially the same as those of Lepper and Vorhes (*loc. cit.*). The description and analyses are given in Table 1.

TABLE 1.—*Materials and methods used*

MATERIAL	DESCRIPTION		SOLIDS	NITROGEN	P <sub>2</sub> O <sub>5</sub>	FAT
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Eggs	1 day old, perfect in appearance	Yolk—	50.37	2.498	1.373	30.89
			50.30	2.507	1.371	30.78
		White—	11.25	1.541	0.04 assumed	
			11.30	1.522		
Oil	Corn oil, Mazola, retail pkg.					
	Cottonseed, retail pkg.					
Salt	C. P. NaCl					
Sugar	Sucrose, U.S.P.					
Spice	Dry mustard, retail pkg.		5.334	2.113		
			5.337			
	Paprika, retail pkg.		2.464	0.765		
			2.436			
Acid	10% acetic for reasons given by Lepper and Vorhes					

The method of preparation of samples was also identical with that used by Lepper and Vorhes, except that a Hamilton Beach electric mixer was used. It was found that it was necessary to add the oil dropwise until the emulsion had formed, and then the remainder of the oil could be added quite rapidly. In the case of Sample 1 the starch was heated with water until a clear suspension was obtained, then cooled and added to the mixer. In future preparation of samples the addition of a gum might prove advantageous as some separation took place after the samples had stood for a considerable length of time.



For the purpose of determining whether or not the oil obtained from the extraction in the sugar determination was pure enough for identification, constants were run on the oil before use in the dressings and collaborators were requested to run these constants on the extracted oils as well as qualitative tests for identity. The results obtained are given in Table 2.

TABLE 2.—*Collaborative results on oil used*

COLLABORATOR	SAMPLE 1				SAMPLE 2			
	A*	B	C	THEORY†	A*	B	C	THEORY†
Halphen test for cottonseed	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.
Index of refraction (25° C.)	1.4691	1.4680	1.4713	1.4692	1.4708	1.4712	1.4729	1.4708
Specific gravity (25°/25° C.)	0.918	0.9055	—	0.919	0.918	0.9133	—	0.919
Iodine number	108.52	104.6	106.0	109.01	126.29	120.0	121.8	128.64

\* Constants run by Floyd Roberts, State Laboratories Dept., Bismarck.

† Represents constants run on oils before use.

### SUMMARY OF RESULTS

The results obtained in the collaborative work are indeed gratifying. Taking into consideration the errors involved in preparation of samples, in sampling, and in analysis, the Associate Referee considers that the methods give deviations from the theory within limits of experimental error.

The acetic acid apparently inverts the sucrose (the extent of inversion varying with time and temperature), and although the methods do not give a true indication of the amounts originally placed in the product during manufacture they do give a true measure of the sugars at the time of analysis.

The constants of the oil were not affected to any great extent, and the figures obtained after extraction indicate that the oil was of sufficient purity for identification. The removal of the last traces of ether seemed to be the most difficult obstacle to overcome, and prolonged heating with agitation from time to time was found to be necessary. Slightly lower figures for the specific gravity and the iodine number indicate that complete removal of ether was not accomplished in all cases.

### ACKNOWLEDGMENT

The Associate Referee wishes to express sincere gratitude to Samuel Alfend, the referee, for his helpful suggestions, and to the following collaborators: B—F. J. McNall, Cincinnati Station, Food and Drug Administration; and C—Ernest M. Hodnett and Vincent E. Stewart, Florida Department of Agriculture. Results obtained by the Associate Referee are listed under Collaborator A.



## RECOMMENDATIONS\*

It is recommended—

(1) That the method for preparation of sample be changed to read as follows:

Before removing any portion of the sample for analysis, transfer to a suitable container, such as a glass fruit jar of somewhat larger capacity than the volume of the sample, and mix until homogeneous with a spatula (2-3 minutes should be sufficient). Repeat mixing before each subsequent portion is removed for analysis if sample has stood for any appreciable length of time. For the various determinations take approximately the quantity directed and weigh. (A suitable weighing bottle was found to be a light 100 ml. flask fitted with a straight glass tube and oversized rubber bulb.)

(2) That the size of sample for the method for total fat be changed to read 1 gram.

(3) That the methods for preparation of sample and total fat, with the recommended changes in (1) and (2), be adopted as official (first action).

(4) That the methods for sugar, and for composition and identification of oil be adopted as official (first action).

(5) That study on salad dressings be continued in respect to methods for determination of such substances as lemon juice, which might affect the  $P_2O_5$  and nitrogen ratio.

## REPORT ON MUSTARD AND MUSTARD PRODUCTS

By J. T. FIELD (U. S. Food and Drug Administration,  
St. Louis, Mo.), *Associate Referee*

It has been found that the present A.O.A.C. method for starch in spices gives high results when applied to mustard, Alfend, *This Journal*, 23, 578 (1940).

Several methods have been published in recent years for the determination of starch in the presence of interfering substances in plants, and modifications of some of these methods were tried by the Associate Referee. The work thus far has been confined to yellow mustard flour and brown mustard flour, with and without added potato starch. The following outline shows the various steps and the results of this investigation.

1.—*Microscopic examination of the samples of mustard flour for natural starch:*

Yellow mustard—no starch detected.

Brown mustard—occasional starch grains.

2. *Analysis of potato starch used:*

	<i>per cent</i>
Moisture ( <i>Methods of Analysis</i> , A.O.A.C., 1940, 211, 2, 3) . . . . .	12.34
Ash, 550° C. . . . .	0.30
Starch (HCl hydrol. av. of 3, factor 0.9) . . . . .	84.67
Protein, fiber, fat (estimated) . . . . .	0.4

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97.71

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 60 (1941).

These results check closely those obtained by Sullivan, *This Journal*, 18, 621 (1935).

3.—*Solution of starch in cold 21 % HCl*, Rask, *Ibid.*, 10, 108 (1927); Pucher and Vickery, *Ind. Eng. Chem., Anal. Ed.*, 8, 93 (1936).—These methods were tried out first because such a solution of the starch would obviate the necessity for volume correction for non-soluble residue, by the use of an aliquot of the filtrate. However the character of the mustard flour made filtration slow and difficult and caused partial hydrolysis and incomplete solution of the starch. The following method and results will illustrate this: A 5 gram sample was extracted with 70% alcohol and ether and dried. The extracted material was treated with 21% HCl at 15°, allowed to stand in the cold 30 minutes with frequent shaking, and filtered with suction. A 50 ml. aliquot was mixed with 150 ml. of 95% alcohol and centrifuged. The clear layer was filtered off and the residue was rinsed with 70% alcohol. The starch residue was inverted by acid in the usual way, and dextrose was determined by the Munson-Walker method.

The results on yellow mustard flour without added starch were negative, while brown mustard flour gave 0.5% starch. Pure potato starch gave a yield of only 45.1%. Yellow mustard flour containing 4% added starch gave recoveries ranging from 36.5 to 54.0%. An attempt was also made to determine starch gravimetrically by weighing the alcohol precipitate. Great difficulty in filtration and washing made this method impractical. Results ranging from 72 to 150% yield were obtained.

4.—*Solution of starch in hot CaCl<sub>2</sub> solution*, Pucher and Vickery and Sullivan, *loc. cit.*—An adaptation of Sullivan's method gave the best results. The method follows:

#### METHOD

##### REAGENTS

(a) *Calcium chloride solution*.—Saturated solution adjusted to approximately 0.025 *N* alkalinity.

(b) *Alcohol*.—95%.

(c) *Alcohol-NaOH solution*.—70 ml. of 95% alcohol + 25 ml. of 0.1 *N* NaOH.

(d) *Ammonium sulfate solution*.—Saturated aqueous solution of C.P. grade (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

(e) *Iodine-potassium iodide solution*.—2 grams I + 6 grams of KI in 100 ml. of water.

##### PROCEDURE

Place a 3–4 gram sample in a 500 ml. Erlenmeyer flask and add 50 ml. of the CaCl<sub>2</sub> solution from a pipet. Connect the flask to a reflux condenser, first wetting the inside of the condenser with 50 ml. of water and draining 1 minute. Boil gently for 15 minutes. Leaving the condenser connected, cool the flask in a pan of water, then add 50 ml. of water from a pipet through the top of the condenser. Remove the flask, stopper, and shake vigorously. Pour the liquid into a centrifuge bottle and centrifuge for about 20 minutes. Pipet 50 ml. of the partly clarified middle layer into a centrifuge bottle containing 150 ml. of the alcohol. Mix, and centrifuge 5 minutes or until clear. Decant the liquid through an asbestos pad into a Caldwell crucible, using suction. Remove the asbestos pad and transfer it to the centrifuge bottle. Rinse particles adhering to the crucible into the bottle with warm water. Add water to approximately 100 ml., shake the bottle vigorously to break up the precipitate, then add 2 ml. of the I-KI solution and 30 ml. of the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution, and mix thoroughly. Centrifuge until clear. Filter with suction through an asbestos pad into a Caldwell crucible and wash the precipitate and the filter with the alcohol-NaOH solution until the blue color is practically gone, then with a few ml. of the

alcohol. Return the pad to the bottle, rinsing the crucible with approximately 20 ml. of water. Add 5 ml. of concentrated HCl and stir to break up the pad. Heat on a steam bath 30 minutes, stirring occasionally. Filter with suction into a clean flask, rinsing the bottle and filter with several small portions of warm water. Rinse the filtrate into a Kjeldahl flask and evaporate to 60–70 ml. by boiling. Attach a reflux condenser and boil for 30 minutes. Cool, rinse into a 100 ml. volumetric flask, nearly neutralize with concentrated NaOH, and make to volume with water. Determine dextrose by *Methods of Analysis*, A.O.A.C., 1940, 504, 48. (Dextrose  $\times 0.9$  = starch.)

The results obtained by the Associate Referee and the collaborators on added starch in yellow mustard flour are shown in Table 1.

TABLE 1.—*Added starch in yellow mustard flour*

ANALYST	MUSTARD	STARCH ADDED	RECOVERY
	grams	grams	per cent
J. T. Field	2.5	0.1000	96.0
	3.0	0.5000	101.2
	3.0	0.2000	97.0
	5.0	0.2000	96.7
	2.5	0.1851	98.4
	3.0	0.2400	98.7
	3.0	0.2400	97.2
S. D. Fine	3.0	0.1851	97.5
	3.0	0.1851	96.8
J. F. Weeks, Jr.	Not reported	6.75%	97.3
	Not reported	6.75%	98.1
	3.0	0.447	100.3
	3.0	0.446	99.4
F. M. Garfield	3.5	0.2061	107.0
	3.5	0.1994	96.7
H. W. Conroy	3.5	0.2000	105.0
	3.5	0.2000	105.6
	3.5	0.2000	105.5
R. Rogers	3.0	0.2076	97.3
Milton Orchin	3.0	Not reported	85.9
	3.0	Not reported	87.2
	3.0	Not reported	83.3
	3.0	Not reported	82.4
A. W. Hanson	Not reported	Not reported	88.9

The method was applied to samples of mustard flour without added starch to determine the influence of interfering polysaccharides or reducing substances. Some results obtained, chiefly on yellow mustard flour, are shown in Table 2.

TABLE 2.—*Reducing substances in mustard flour*

ANALYST	MUSTARD SAMPLE	STARCH (CALCULATED)
	<i>grams</i>	<i>per cent</i>
J. T. Field	5	0.00
	5	0.00
	3	0.02
	2.5	0.03
	2.5	0.10
	2.5	0.05
	2.5	0.19*
	5	0.50*
F. M. Garfield	3.6666	0.16
	3.7814	0.10
Milton Orchin	3	0.01
	3	0.01
H. W. Conroy	3.5	0.12
	3.5	0.14
A. W. Hanson	Not reported	0.00
R. Rogers	3.0	0.00

\* Brown mustard flour.

Attempts to shorten the method by eliminating the iodine precipitation and washing repeatedly with dilute alcohol gave "recoveries" from 112 to 170 per cent.

#### SALT IN MUSTARD

A modification of the open Carius method, *Methods of Analysis*, A.O.A.C., 1940, 301, 115, was applied to mustard flour containing an added amount of standardized salt solution. The following method was used:

#### TOTAL CHLORIDES IN PREPARED MUSTARD

Weigh 5 grams of prepared mustard from a weighing bottle, place in a 300 ml. conical flask, and add an excess of standard 0.1 *N* AgNO<sub>3</sub> (usually 30 ml. is sufficient). Mix thoroughly and then add 15 ml. of HNO<sub>3</sub>. Bring to boiling on a hot plate. Add to the boiling mixture 15 ml. of 5% KMnO<sub>4</sub>, 5 ml. at a time, rotating the flask after each addition to mix the contents. Add approximately 50 ml. of water and filter into a 200 ml. volumetric flask. Wash the filter until the filtrate is free of AgNO<sub>3</sub> and make up to the mark with water. Mix thoroughly and titrate a 100 ml. aliquot with 0.1 *N* KSCN, using 2 ml. of a saturated solution of ferric alum as an indicator. Calculate the chlorides as NaCl.

The results are given in Table 3. (All samples contained approximately 2 grams of mustard flour.)

TABLE 3.—*Total chlorides in prepared mustard*

ANALYST	NaCl ADDED	NaCl RECOVERED	CORRECTION FOR BLANK	RECOVERY
	mg.	mg.		per cent
J. T. Field	None	0.6	0.6*	—
	None	1.3	1.3	—
	None	0.5	0.5*	—
	None	1.1	1.1	—
	116.9	117.5	0.6*	100.0
	116.9	118.1	1.3	99.9
	118.5	118.2	0.5*	99.3
	118.5	118.9	1.1	99.4
S. D. Fine	None	0.8	0.8	—
	None	0.2	0.2	—
	118.5	118.2	0.5	99.3
	118.5	118.8	0.5	99.8
F. M. Garfield	None	1.3	1.3	—
	116.9	118.2	1.3	100.0
	116.9	118.3	1.3	100.1

\* Brown mustard.

For comparative purposes determinations were also made by the official method for chlorine in plants, *Methods of Analysis, A.O.A.C.*, 1940, 134, 34, 36, 37, on the same samples. The results are given in Table 4.

TABLE 4.—*Chlorides obtained by A.O.A.C. method*

ANALYST	NaCl ADDED	NaCl RECOVERED	CORRECTION FOR BLANK	RECOVERY
	mg.	mg.		per cent
J. T. Field	None	1.3	1.3	—
	None	1.4	1.4	—
	118.5	119.5	1.3	99.7
	118.5	119.5	1.4	99.7

## SUMMARY

The double precipitation method of Sullivan for the determination of starch was found to give more promising results than did any other method tried. It appears to eliminate interfering substances and to yield a recovery of starch of about 97 per cent. Difficulties with the method were experienced by some collaborators, and some simplifications may be necessary.

The open Carius method employing digestion with nitric acid in the presence of silver nitrate was applied to the determination of salt in mustard. The results obtained compare well with the recovery by the alkaline ash method. The method is more rapid and simple than the ashing method.

## RECOMMENDATIONS\*

It is recommended—

(1) That additional collaborative work be done on the determination of starch in mustard flour and prepared mustard and that the revisions of the official method suggested by the Associate Referee be used.

(2) That the method for the determination of salt in mustard given in this report be adopted as tentative.

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REPORT ON FISH AND OTHER MARINE PRODUCTS

By H. D. GRIGSBY (U. S. Food and Drug Administration,  
Philadelphia, Pa.), *Referee*

In accord with recommendations approved at the last meeting, a review of the methods for meat and meat products in Chapter XXVIII of *Methods of Analysis, A.O.A.C.*, 1940, was made to determine whether or not they are applicable to fish and other marine products.

The Referee considered that since meat and fish are closely related foods, most of the methods now official or tentative for meat and meat products should be satisfactory for fish, and if so, then in the newer chapter it should be possible to cover these determinations by reference. A study of the meat methods confirms this opinion.

With certain exceptions, the methods listed in Chapter XXVIII under numbers 7 to 37, inclusive, should be equally applicable to fish. A few general comments follow.

*Paragraph 8.*—This method is now included in the fish chapter by reference.

*Paragraph 9.*—The Folin aeration method for the determination of ammonia nitrogen may require some modification to make it suitable for fish, as pointed out by Clark, *This Journal*, 4, 231 (1917), who recommended Steel's modification<sup>1</sup> because of the possible presence of  $MgNH_4PO_4$  in fish in the early stage of deterioration. Clark also suggests that amines in fish, if present, will be carried over with the ammonia.

*Paragraphs 24, 25, and 26.*—These methods, given as references to the general methods for preservatives, metals and coloring matter, are equally applicable to fish.

*Paragraphs 27–35 inclusive.*—These methods for the determination of various nitrogenous fractions or compounds in meat should apply equally well to fish.

The Referee does not believe that collaborative study of the applicability of these methods to fish is warranted at this time for the sole purpose of obtaining official approval by the Association.

It is quite possible that results by some of these methods might be useful as data on changes in fish undergoing decomposition and thus serve as indices of such decomposition. There are several references in the literature to the use of ammonia nitrogen for this purpose, and for related products, such as eggs, this determination is frequently used.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 60 (1941).

<sup>1</sup> *J. Biol. Chem.*, 8, 365 (1910).



Hillig and Clark, *This Journal*, 21, 688 (1938), have proposed a method for the determination of volatile acids and formic acid in canned salmon and tuna fish, as a useful index of the decomposition in these products, and have accumulated some supporting data to show the increase in these acids as decomposition advances.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method proposed by Hillig and Clark for the determination of volatile acids and formic acid in canned salmon and tuna fish be studied collaboratively.

(2) That the method for the determination of ammonia nitrogen be studied collaboratively.

(3) That the recommendations offered by Associate Referee Tubis for further work on fat and moisture in fish be approved.

(4) That methods for ash, salt, and total nitrogen, now official—first action, be studied further before final adoption is recommended.

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### REPORT ON FAT AND MOISTURE IN FISH

By MANUEL TUBIS (U. S. Food and Drug Administration,  
Philadelphia, Pa.), *Associate Referee*

The determination of moisture and fat in fish is more difficult than the same determination in meat or other food products because of the characteristics of fish flesh and the highly unsaturated character of the fish oils.

When the usual methods of drying are applied to some types of fish the result is a hornlike crust that retards the rapid removal of the water, and the extended time required hastens the oxidation of the fat and prevents its subsequent extraction.

These difficulties have been recognized by other workers (1, 2, 3), and they have proposed methods of obviating them.

The problem may be resolved into several principal parts; namely, the determination of moisture only, the determination of fat only, and the determination of moisture and fat simultaneously or consecutively. Additional problems are the choice of a solvent that will extract the fat and no other extractives (1) and the extraction of oxidized fat such as occurs in fish meal (1). The solvents that have been used include saturated hydrocarbons like petroleum benzin and benzene, chlorinated hydrocarbons like dichlorethylene, ethers like ethyl and isopropyl, ketones like acetone, and the cyclic ether 1,4 dioxane (2). At the present time no solvent is known that will extract all the fat regardless of its state of oxidation (4).

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\* For report of subcommittee and action by the Association, see *This Journal*, 24, 58 (1941).

Some workers have "assumed that the oil content of a substance is equivalent to the content of substances extracted by ethyl ether from the dried material" (3). Agreement upon some such an assumption by workers in this field should simplify the problem.

The proposed methods for the determination of moisture range from drying the sample in a vacuum desiccator (5) to drying in an oven at 130°C. (6). The Associate Referee has investigated a method for simultaneous drying and fat extraction, and the use of isopropyl ether in the apparatus of Kaye, Leibner and Connor (7).

This preliminary study was undertaken to compare the various proposed methods for fat and moisture and to select those that can be completed in a reasonable time and also give reproducible results of sufficient accuracy. Fat and moisture determinations were made on one sample of canned salmon and one sample of fresh mackerel. No study was made of frozen, salted, or dried fish or fish meal in which considerable decomposition or oxidation of the fat may have taken place.

There follows a description of the samples, method of preparation, and of the method used in the drying and/or extraction of the fat. The results are given in Tables 1 and 2.

The salmon sample, with all the accompanying liquid, was ground twice in a food chopper and placed in a closed jar. Before part of the sample had been removed, the entire contents were freshly mixed.

The skin, bones, and viscera of the mackerel were removed, the edible portions were ground twice in a food chopper, and a procedure was followed that was exactly like that used on the salmon. The problem of obtaining a homogeneous sample of fresh fish is complicated by the tendency of the oil and water to separate, which makes necessary thorough mixing of the sample before the aliquot is removed.

#### DETERMINATION OF MOISTURE

The following method was used to prepare the samples for drying when fat extractions were to be made subsequently.

Fit a lead-foil dish into a covered aluminum drying dish 70 mm. in diameter by 33 mm. high. Place in the lead dish 20 grams of dried sand and a 1.5 inch glass rod with a flattened end and dry for 1 hour at 100°–110°C. Cool, and weigh. Add to the dish about 5 grams of the freshly mixed sample, quickly cover, and weigh immediately. Uncover the dish, thoroughly mix the sample with the sand, and leave the rod in the dish so that its upper end remains clean. After appropriately drying the sample (see below), cool, and weigh. Break up the dried mass by means of the rod and transfer quantitatively to an extraction thimble; then roll the dish with its adhering material and place with the rod in the thimble.

The methods of drying that follow will be referred to hereinafter by the number assigned.

*Method 1.*—Sample dried in a vacuum oven at the boiling point of water at less

than 25 mm. pressure to constant weight (approximately 5 hours). These conditions are usually specified for drying other food products.

*Method II.*—Harrison's procedure (2) of drying the sample in a vacuum oven at 80°C. for 2 hours, which is only to prepare the sample for fat extraction, and not to be used as a method for determining moisture.

*Method III.*—The sample is dried in a vacuum desiccator over freshly boiled  $H_2SO_4$  to a minimum weight (8). The sample is weighed at the end of 24 hours and thereafter at each 24 hour interval. It was found that after a minimum weight had been reached, the sample began to gain weight even though kept in a desiccator. Duplicates reached minimum weights at different times, indicating uneven rates of drying despite adequate division of the sample. The time required for "complete" desiccation was 6 days in the case of salmon, and 3 days for the mackerel. This time may extend to 2 weeks for some samples. The Associate Referee considers that as a standard or reference method, this one is very time-consuming.

*Method IV.*—The dishes containing the prepared samples are dried at the temperature of boiling water in a vacuum oven, which is then swept through with  $CO_2$  several times, and the heating is continued for 5 hours in a slow current of the gas. It is believed that the inert atmosphere prevents oxidation of the fish oils during the drying period.

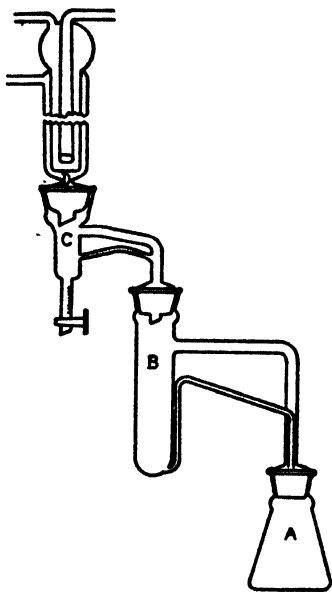


FIG. 1. APPARATUS FOR CONTINUOUS DRYING AND EXTRACTION OF BIOLOGICAL MATERIALS

*Method V.*—This method, proposed by Veshchezerov (6), specifies drying the sample preliminarily at 60°–80°C. for 1–2 hours to prevent the formation of crusts and then drying at 130°C. for 0.5–1 hour. The directions do not specify the use of sand for increasing the drying surface. Duplicate determinations were made on one salmon sample by heating at 60°, the lower temperature for the minimum time (1 hour) and at 130°, the higher temperature for the minimum time (0.5 hour). The other sample was heated at 80° for 1.5 hours and at 130° for 1 hour. The first sample showed a moisture content somewhat lower than that obtained by the other methods, while the second agreed closely with the results by the other drying methods. The results of an analysis of the same sample at a later date agreed well with duplicate samples dried in a vacuum oven for 5 hours as directed in Method I.

Triplicate analyses were made of the mackerel. The sand used and the mixing were as previously described in two cases, but the sand was omitted in the third case. The result was a lower percentage of moisture for the last analysis, when all samples were heated for identical periods (Table 2).

The Associate Referee considers that Method V, because of its simplicity and rapidity, should be investigated further for use where moisture only is to be determined, since the heating to 130°C. may cause some decomposition of the oil present.

*Method VI.*—This method combines a simultaneous drying and fat extraction, and isopropyl ether is used in the apparatus of Kaye, Leibner and Connor (7) as shown in Figure 1.

The apparatus was used in the following manner:

Whatman thimbles, inside length 50 mm., inside diameter 19 mm., are extracted for 2 hours with isopropyl ether, dried to remove the solvent, then dried in an oven at 100°–110°C. for 2 hours. The thimble is then cooled and weighed in a stoppered tube or weighing bottle. An alundum extraction thimble, the Norton RA98 Coarse 19×90 mm. may be used. About 5 grams of the freshly mixed sample is added to the thimble, which is replaced in its weighing tube and quickly reweighed. The weight of flask A is recorded. The thimble is placed in extraction tube B and the apparatus is connected, except for condenser D. Anhydrous isopropyl ether, distilled over Na, boiling point 67.5°C., is now added through the top of C until siphoning occurs through the side arm of B. The condenser is connected, and water baths are placed around A and B, heated to 80°C., and kept at this temperature for 7 hours. The time required for the removal of the water may vary from sample to sample so that the volume should be noted and the temperature maintained as long as is necessary. After all the water is separated, the temperature of the bath around B is lowered to 60°C. and extraction is continued 6 hours longer. After the volume of water has been noted the stopcock is opened and the solvent is distilled out by raising the temperature of the water bath around B to boiling. The apparatus is disconnected, and flask A is removed, cooled, and weighed, the last traces of solvent being removed by applying a slight vacuum. The isopropyl ether extractives may be purified by dissolving in anhydrous ethyl ether and, if any insoluble residue remains, filtering through a sintered glass funnel, washing with small portions of ether, and evaporating the ether. The fat is then dried at 80°C. in a vacuum. If no residue is found, the ether is removed and the flask is dried and weighed. The weight of dried residue can be obtained by weighing the extraction thimble. It was found that a total extraction period of 13 hours was sufficient and that no further water separated after 7 hours. An additional 3 hour extraction yielded no further extractives.

The figure given in Table 2 for moisture by Method VI was determined by difference and really represents all the volatile matter, including the moisture. This method has the following advantages: Moisture and fat are determined simultaneously on the same sample; volatile substances other than water are not lost in this drying procedure; the temperature of drying is low, 67.5°C., the boiling point of the solvent; and the air is excluded by displacement with isopropyl ether vapor, the two last factors combining to lessen the possibility of decomposition and oxidation. The fat content obtained by this method (Table 2) was lower than that obtained by acetone extraction of the other samples dried by the other methods. The fat obtained by isopropyl extraction is entirely liquid, with a light yellow color showing no carbonization and having no burnt or "smoky" odor, while that of the acetone extraction, as finally weighed, is dark, has a very slight odor of decomposition, and is similar in appearance to that obtained by the semi-quantitative method of Stansby and Lemon (3).

The Associate Referee considers that this method offers possibilities, and he will investigate it further.

#### DETERMINATION OF FAT

For the fat determinations, the samples that had been dried by the other

methods were quantitatively transferred to Whatman thimbles with the lead dish, sand, and rod and extracted for 16 hours with acetone in an all-glass Soxhlet apparatus. The acetone was removed on the steam bath at a low temperature, and the residue was dissolved in anhydrous ethyl ether and filtered through a sintered glass funnel, which was then washed with small portions of ether. The ether was removed at a low temperature, and the fat was dried at 100°C. for 45 minutes, and weighed. Reheating for another 45 minutes resulted in a loss of weight of 2 parts per 1000.

In the "semiquantitative method" of Stansby and Lemon (3), in which the oil is extracted by anhydrous ethyl ether and anhydrous sodium sulfate by shaking for 1 hour, it is assumed that the fish flesh and sulfate are mixed thoroughly before the ether is added. If this is not done, the flesh forms a single mass and incomplete extraction results. The procedure followed here was to weigh the 20 gram sample into stoppered centrifuge bottles. The sodium sulfate was then added and thoroughly mixed, and the glass rod used was later cut and dropped into the bottle. The measured quantity of ether (100 ml.) was added, and the bottle was rotated on a vertical wheel for 1 hour. Access to the entire mass of flesh by the ether was obtained. A 20 ml. aliquot was removed, filtered, evaporated, dried at 100°C. for 1 hour, and weighed. The character of the oil finally ob-

TABLE 1.—*Canned salmon*

METHOD OF DRYING	TIME	MOISTURE	FAT
	hours	per cent	per cent
I.—Vacuum oven at boiling point of water at pressure of less than 25 mm.	5	70.80 70.76	5.56
II.—Vacuum Oven at 80°C., at pressure of less than 10 mm.	2	70.60 70.56	
III.—Vacuum desiccator at room temp.	144	70.60 70.50	
IV.—Oven at boiling point of water in a current of CO <sub>2</sub>	5	70.80 70.74	— 5.63
V.—Veshchezerov's method Electric Oven	$\left. \begin{array}{l} 60^{\circ}\text{--}80^{\circ}\text{C.} \\ 130^{\circ}\text{C.} \end{array} \right\} \begin{array}{l} 1 \\ 0.5 \end{array} \text{ } ^a$ $\left\{ \begin{array}{l} 60^{\circ}\text{--}80^{\circ} \\ 130^{\circ} \end{array} \right. \begin{array}{l} 1.5 \\ 1 \end{array} \text{ } ^b$ $\begin{array}{l} 74^{\circ} \\ 130^{\circ} \end{array}$	68.25 70.79 70.72 70.59	5.31

<sup>a</sup> The minimum time specified.

<sup>b</sup> The maximum time specified.

tained was entirely comparable with that obtained by the Kaye, Leibner, and Connor apparatus. The results by this method are shown in Table 2.

A portion of the mackerel sample extracted with 1,4 dioxane without preliminary drying gave a larger percentage of extractive than did any of the other solvents. This is in agreement with the findings of Harrison and may be accounted for by the higher temperature necessary for the extraction and removal of the solvent, at which temperature materials other than fat may have been extracted and the rate of oxidation of the fat accelerated.

TABLE 2.—*Fresh mackerel*

METHOD OF DRYING OR EXTRACTION	TIME	MOISTURE	FAT
	<i>hours</i>	<i>per cent</i>	<i>per cent</i>
I.—Vacuum oven at boiling point of water at pressure of less than 25 mm.	5	61.59 61.32	19.60 <sup>a</sup>
II.—Vacuum oven at 80°C., at pressure of less than 10 mm.	2	60.67 60.72	20.31 <sup>a</sup>
III.—Vacuum desiccator at room temp.	72	60.43 60.15	
IV.—Oven at boiling point of water in a current of CO <sub>2</sub>	5	60.15 60.52	— 19.67 <sup>a</sup>
V.—Veshchezerov's method { 60–80° Electric Oven { 130°C.	2 } 1 }	61.05 61.05 60.61 <sup>b</sup>	
VI.—Extraction apparatus of Kaye, Leibner, and Connor	7	61.71 <sup>c</sup>	19.02 <sup>d</sup>
Semiquantitative method of Stansby and Lemon			18.88 18.92
Soxhlet extraction on undried sample			20.45 <sup>e</sup>

<sup>a</sup> Acetone extraction.

<sup>b</sup> Dried without mixing with sand as described.

<sup>c</sup> Calculated from dried residue plus extracted fat.

<sup>d</sup> Solvent used was anhydrous isopropyl ether.

<sup>e</sup> Solvent used was 1, 4 dioxane.

It is recommended\* that further study and collaborative work be done on the Veshchezerov method if moisture only is to be determined, on the method of Stansby and Lemon for a rapid semiquantitative method for fat, and on the method of Kaye, Leibner, and Connor for moisture and fat.

\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 58 (1941).

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## REPORT ON CACAO PRODUCTS

By. W. O. WINKLER (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The work this year was directed mainly toward obtaining new and better methods for the determination of three materials present in or added to cacao products, namely, lecithin, milk protein, and pectic acid. The first two materials are added as such or in the constituents of chocolate products. The pectic acid is a measure of the shell content of the nibs or the liquor used. In addition to the investigations on these materials, some additional work was done on the method described in last year's report for the determination of cacao shell in cacao nibs, *This Journal*, 23, 593 (1940). The method was published in the 1940 edition of *Methods of Analysis*, A. O. A. C. and in the Federal Register, Vol. 5, No. 205, page 4152.

Some work was also done on the determination of the dry, fat-free, chocolate mass in cacao products.

## LECITHIN

Lecithin, obtained mainly from the soy bean, is added quite extensively to chocolate products, especially to the coatings. In order to test methods for the detection of this substance, the Associate Referee undertook collaborative study of two methods. The methods were taken from *This Journal*, 14, 537 (1931) and were designated as III (a) and V (a). Two samples (A and B) of the same chocolate liquor, with and without added lecithin, were subjected to analysis by the two methods. Twelve collaborators reported on Method III (a) and eleven on Method V (a). The percentage of added lecithin in Sample B was obtained by subtracting the percentage of lecithin found in Sample A (no lecithin added) from the percentage of lecithin found in Sample B (lecithin added).

The results varied considerably among the different collaborators, but the average quantity of lecithin obtained by all the collaborators by both methods was approximately the amount added. To Sample B was added 0.27 per cent of lecithin, while the average amounts obtained by the

collaborators were 0.24 and 0.26 per cent by Methods III (a) and V (a), respectively. Because of the variation mentioned, it is believed the subject should be studied further.

### MILK PROTEIN

The Associate Referee on Milk Protein investigated the method given in the 1938 report of the referee, *This Journal*, 22, 603 (1939). Additional analyses of skim milk and whole milk powders confirmed the approximate accuracy of the factor 1.07 used to obtain the total protein from that precipitated by the reagents used (casein and albumin).

The associate referee reported collaborative results on two samples by the present tentative method and the proposed method referred to above. Results by the two methods agree quite well on Sample A. Sample B contained a fairly high percentage of milk protein for a milk chocolate (considerably higher than A), and several low results were obtained on this sample by the proposed method. The Referee is of the opinion that this was due to insufficient acid in the nitrogen digestion in the presence of selenium as a catalyst. Although selenium accelerates the digestion, there have been other reports in the literature of the loss of nitrogen with its use if considerable excess acid was not present, or if digestion was prolonged. Low results were obtained by the referee on Sample B, using the proposed method and making use of the mixed catalysts mercury and selenium in the digestion.

When the determination by the proposed procedure was repeated and the use of selenium in the catalyst was omitted, results very near the theoretical were obtained. It is believed therefore that the reason given accounts for the cases where low results were obtained. Further work should be done on the methods.

### CACAO SHELL BY PECTIC ACID METHOD

Work on this determination was continued. Two collaborative samples were sent to a number of collaborators. Sample 1 was a sweet chocolate containing, on the sample basis, 1.96 per cent of added shell powder. The sample contained, before the addition of the powdered shell, 19.15 per cent of fat-free dry cacao mass, so that the shell added was equal to 9.45 per cent of the total dry fat-free cacao mass. Sample 2 was a milk chocolate to which an amount of dry fat-free powdered cacao shell equal to 1 per cent of the weight of the milk chocolate was added. This constituted 11.38 per cent of the total fat-free dry chocolate and shell mass.

Collaborators were instructed to make the determinations by the method submitted.

The details of the method are not given here, since further revision may be necessary in view of the fact that the collaborative results are not entirely satisfactory. In outline the method is as follows:



The sample is extracted with ether or petroleum benzin by the centrifugal method to remove fat and the solvent in the residue is expelled. Products containing milk solids are then extracted by shaking with water and ammonium oxalate followed by centrifugation to remove the extract. This step is omitted in products containing no milk or dairy ingredients. The residue is extracted similarly in succession with acidified 80% alcohol, alcohol, and finally with ether. The residue is then dried and weighed to obtain the fat-free dry cacao mass in the sample. Pectin in the residue is extracted by digesting the material with about 0.25% ammonium oxalate solution for 3 hours on the steam bath followed by centrifugation.

The extracts (after combination with the first oxalate extract from which milk protein has been precipitated in case of products containing dairy ingredients) are evaporated and the pectin precipitated with acidified alcohol. After the precipitate has been dissolved, some impurities are filtered off on a filtercel filter. The filtrate is saponified and pectin is precipitated as pectic acid (digalacturonic) with HCl. The precipitate is filtered, washed, dried, weighed, etc.

Collaborative results obtained by the method on the samples are given in Table 1.

TABLE 1.—*Per cent pectic acid in fat-free, dry cacao mass*

COLLABORATOR	SAMPLE 1	SAMPLE 2
	SWEET CHOCOLATE	MILK CHOCOLATE
1	0.48; 0.51	0.58; 0.63
2	0.45; 0.51	0.47; 0.54
3	0.46; 0.45	0.59; 0.54
4	0.11; 0.15	0.37; 0.40
5	0.26; 0.28	0.19; 0.21
Calculated pectic acid from shell contains 4.25% and liquor .06%	0.45	0.53

Of the five collaborators on Sample 1, three agree very well, and all are close to the theoretical percentage. The results obtained by the other collaborators are low, indicating a loss of some pectin at some step in the method.

Of the five collaborators on Sample 2, three show very good agreement, while one is a little low and the other considerably below the theoretical percentage, again indicating some loss of pectin. The Referee believes the low results obtained may be due to incomplete solution of the alcohol precipitate or to insufficient washing of the filter used to remove interferences contained in the alcohol precipitate. A slightly ammoniacal solution of the alcohol precipitate containing the pectin is filtered on a Büchner funnel through a paper overlaid with filtercel. The filtercel has considerable adsorption power for interferences and tends somewhat to adsorb pectin. Filtration is inclined to be slow and if the filter is not washed well, low results may be obtained. Some revision in this part of the procedure may be necessary.

## RECOMMENDATIONS\*

It is recommended—

(1) That work on the determination of lecithin in cacao products be continued.

(2) That the proposed method for milk protein in milk chocolate be given further collaborative study with the use of mercury alone as catalyst.

(3) That further collaborative work be done on the method for pectic acid in cacao products.

(4) That the procedure given in the report on chocolate in sweet and milk chocolate be adopted as tentative for the determination of the chocolate constituent of sweet chocolate (with or without added dairy ingredients), milk chocolate, mixed milk chocolate, skim milk chocolate, buttermilk chocolate, cocoa and fat (other than cacao fat), coatings, and chocolate and fat (other than cacao fat) coatings.

## REPORT ON MILK PROTEINS IN MILK CHOCOLATE

By MARIE L. OFFUTT (U. S. Food and Drug Administration,  
New York, N. Y.), *Associate Referee*

The Referee on Cacao Products last year proposed a modification of the present tentative method that would shorten the time and also speed up the digestion, which was slow because of the incessant foaming in the beginning. Some work on skimmed milk powders had been done so that the factor 1.07 for the new method was suggested. Further work on more milk powders this year agreed with this 1.07 factor.

Two samples of milk chocolate were sent to collaborators for the determination of their milk protein by the present A.O.A.C. method, *Methods of Analysis, A.O.A.C.*, 1935, 198, and by the proposed modified method, *This Journal*, 22, 603 (1939). The chemists reporting and to whom acknowledgment is made are the following, connected with the U. S. Food and Drug Administration: J. H. Loughrey, Boston; Maryvee G. Yakowitz, San Francisco; J. H. Bornman, Chicago; J. W. Cook, Seattle; W. O. Winkler, Washington; H. Shuman, Philadelphia; and W. T. Mathis, Agricultural Experiment Station, New Haven, Conn.

All the collaborators agreed that the proposed method was much faster and did not require the constant attention during digestion that the present A.O.A.C. method does. Several chemists also called attention to the fact that only one determination was necessary instead of the two specified in the present method.

Mathis called attention to the one determination and stated that this determination reduced the normal manipulative error and gave more accurate results.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 62 (1941).

*Collaborative results*  
(Total Milk Protein (%))

COLLABORATOR	SAMPLE A—CALCULATED 3.24		SAMPLE B—CALCULATED 5.99	
	A.O.A.C. METHOD	PROPOSED METHOD	A.O.A.C. METHOD	PROPOSED METHOD
W. T. Mathis	3.54	3.80	5.81	5.93
	3.60	3.77	5.84	6.00
J. H. Loughrey	3.33	3.66	5.71	5.36
	3.30	3.60	6.00	5.34
Maryvee G. Yakowitz	3.52	3.81	6.11	5.99
	3.41	3.79	5.97	5.81
	3.52	3.71	5.92	5.81
	3.48	3.66		5.91
		3.69		5.83
J. W. Cook	3.36	3.77	5.66	5.80
	3.60	3.69	5.58	5.90
	3.29			
	3.40			
J. H. Bornmann	3.76	3.79	6.19	5.96
	3.91	3.75	5.96	5.94
M. L. Offutt	3.74	3.55	6.26	5.84
	3.74	3.52	6.38	5.79
	3.84	3.52		5.83
W. O. Winkler	3.85	3.63	6.00	5.95
	3.87	3.65	6.01	5.89
H. Schuman	3.23	3.36	5.61	5.04
	3.43	3.41	5.75	4.35
				3.21
				3.37

Two of the chemists considered that the amount of paper pulp to be used should be stated more definitely and suggested that one 15 cm. circle of No. 1 Whatman pulped should be sufficient. The results on Sample A, which contained less milk solids, are in fairly close agreement among the analysts, but there seems to be more variation on Sample B, with higher milk solids in several cases. It is believed, however, that by further work this variation may be overcome. It is therefore recommended that further work be done on this proposed method for the determination of milk proteins in milk chocolate.

## REPORT ON LECITHIN IN CACAO PRODUCTS

By J. H. BORNMANN (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

A contributed paper by Winkler and Sale, *This Journal*, 14, 537 (1931), on the "Detection of Lecithin in Chocolate Products," gives a number of analytical procedures for the extraction and estimation of lecithin. Two of these methods, III(a) and V(a), were selected for collaborative study.

A sample of commercially ground Superior Bahia liquor was sent to collaborators (Sample A) and also a portion of the same liquor to which had been added 0.3 per cent of lecithin (Sample B). The lecithin was purified by dissolving in petroleum benzin, filtering, and evaporating the solvent. It was found to contain 90 per cent of lecithin. This lecithin was gradually mixed with the melted liquor by prolonged stirring until a smooth mixture was obtained with about 100 grams of the liquor, which was then incorporated by further thorough mixing with the bulk of the liquor. Sample B thus contained 0.27 per cent of added pure lecithin.

It was suggested to collaborators that 10 grams of melted sample be taken for analysis, and that the weighing and ashing of lipoids be done by the method given in the 1935 edition of *Methods of Analysis*, A. O. A. C., p. 300, rather than the 1925 edition, and that the entire extract be used. The use of a platinum dish rather than a Pyrex beaker was optional.

The following collaborators reported:

- F. X. Kobe, Rockwood & Company, Brooklyn, N. Y.
- E. W. Meyers, Hershey Chocolate Co., Hershey, Pa.
- R. P. Mears and Associates, Walter Baker & Co., Inc., Dorchester, Mass.
- Joseph Stanley, American Lecithin Co. Inc., Chicago, Ill.
- F. E. Carruth, Maywood Chemical Works, Maywood, N. J.
- S. D. Fine, U. S. Food and Drug Admin., St. Louis, Mo.
- M. L. Offutt, U. S. Food and Drug Admin., New York, N. Y.
- H. W. Conroy, U. S. Food and Drug Admin., Kansas City, Mo.
- J. H. Bornmann, U. S. Food and Drug Admin., Chicago, Ill.
- J. H. Loughrey, U. S. Food and Drug Admin., Boston, Mass.

## COMMENTS OF COLLABORATORS

F. X. Kobe.—In Method III(a) the addition of alcohol in the first extraction resulted in a visible coagulation of the protein material, and the extract itself was a clear solution. Keeping the extracted material in contact with alcohol preliminary to the second extraction causes some of the coloring material to dissolve. Unless the fat and other lipoids are thoroughly freed from this alcohol some of these cacao colors will still remain dissolved and will cause inaccuracies in the total lipid content. In the filtration of the dried extract after re-solution in  $\text{CHCl}_3$  the pledget of cotton must be carefully placed in the filtering funnel in order to prevent solid material from passing through and still maintain a fairly steady rate of flow in the filtering process. The procedure from this point, if followed carefully, presents no difficulties.

In Method V(a) the absence of ethyl alcohol in the first extraction with petro-

TABLE 1.—*Collaborative results (%)*

ANALYST	SAMPLE A—LIQUOR			SAMPLE B LIQUOR+LEICITHIN			ADDED LEICITHIN FOUND
	LIPIDS	P <sub>2</sub> O <sub>5</sub>	LEICITHIN	LIPIDS	P <sub>2</sub> O <sub>5</sub>	LEICITHIN	B-A

*By Method III(a)*

1	54.15		0.26	54.36		0.43	
	54.08		0.27	54.29		0.46	0.18
2	55.22	0.0304	0.35	55.33	0.0480	0.55	
	55.23	0.0311	0.35	55.66	0.0481	0.55	0.20
3	54.98	0.0261	0.30	55.51	0.0470	0.53	
	54.99	0.0213	0.24	55.75			0.26
4	58.30	0.0279	0.32	58.63	0.0477	0.54	
	1		1				0.22
5	54.74	0.0370	0.42	54.86	0.0584	0.66	0.24
6	55.31	0.013	0.15	55.64	0.046	0.52	0.37
7			0.50*			0.79 <sub>2</sub>	0.29
			0.64 <sup>3</sup>			0.84 <sub>3</sub>	0.20
8	54.69	0.029	0.33	55.38	0.051	0.58	
	54.75	0.030	0.34	55.12	0.049	0.56	0.24
9	52.96	0.0310	0.35	55.15	0.0447	0.51	
	53.34	0.0295	0.33	54.13	0.0436	0.50	0.17
10	54.71	0.0267	0.30	54.92	0.0357	0.41	
	54.63	0.0272	0.31	54.92	0.0360	0.41	0.11
11	54.62	0.0262	0.30	55.21	0.0554	0.63	
	54.57	0.0322	0.37	55.12	0.0553	0.63	0.30
12	54.48	0.011	0.13				
	53.83	0.012	0.13	55.06	0.040	0.45	0.32

*By Method V(a)*

1	54.21		0.28	54.50		0.48	
	54.19		0.29	54.38		0.49	0.20
2	55.24	0.0413	0.47	54.64	0.0666	0.76	
	55.13	0.0423	0.48	54.42	0.0659	0.75	0.28
	55.68	0.0326	0.37	55.69	0.0465	0.53	0.16
3	55.39	0.0368	0.42	55.46	0.0532	0.60	0.18
4	59.06	0.0368	0.42	58.98	0.0581	0.66	0.24
	1		1				
5	54.83	0.0357	0.41	55.64	0.0573	0.65	0.24
8	54.98	0.038	0.43	55.37	0.061	0.69	
	55.22	0.040	0.45	55.50	0.061	0.69	0.25
9	55.83	0.0332	0.38	55.72	0.0531	0.60	
	55.87	0.0320	0.36	55.80	0.0562	0.62	0.24
	54.84	0.0311	0.35	55.15	0.0543	0.62	
10	54.82	0.0306	0.35	55.19	0.0534	0.61	0.27
	54.95	0.0307	0.35	55.08	0.0542	0.62	
11	54.68	0.0343	0.39	55.08	0.0598	0.68	
	54.55	0.0328	0.37	54.93	0.0598	0.68	0.30
12	55.09	0.012	0.13	54.68	0.056	0.63	
	54.22	0.017	0.19	55.29	0.057	0.65	0.48

<sup>1</sup> Dried 2½ hours. Not taken to minimum weight.<sup>2</sup> 5 hour Soxhlet extraction with hexane.<sup>3</sup> 7 hour Soxhlet extraction with hexane.

leum benzin results in a cloudy extract. (It is for this reason that the practice has been adopted in our laboratory to add a few grams of sugar to a liquor sample before determining the fat content. The extracts are clearer and result in more accurate determinations.) The proteins that produce this clouded extract are later precipitated by the subsequent addition of alcohol, but this precipitation takes place in the beaker containing the extracted lipoids. As a result more extraneous material accompanies these lipoids and tends to cause more spattering. Since this first extraction represents the only deviation from Method III(a) except in the use of solvent, it may explain the slightly higher results obtained in Method V(a). Either some of the protein material is carried through, or it undergoes a more thoroughgoing extraction in the beaker containing the extracted fat.

E. W. Meyers.—Had some difficulty in getting a clear  $\text{CHCl}_3$  extract, Method V(a). For that reason, 15 ml. of additional alcohol was added to coagulate the nitrogenous matter before centrifuging. (His first two results, V(a), were obtained with that procedure.) In subsequent determinations, the pledget of cotton was tightened sufficiently to retain all insoluble matter, and vacuum was used to hasten filtration. (The third result on V(a) and both results on III(a) were thus obtained.) For lack of time, it was necessary to hasten our fat determinations so that the weight readings did not quite reach a constant point.

R. P. Mears.—The  $\text{CHCl}_3$  filtrate came through cotton pledget clear on all samples, but seemed to develop a cloudiness as washing continued. On evaporation of  $\text{CHCl}_3$ , there appeared to be a non-fatty material in the residue. Therefore, residue was re-dissolved in  $\text{CHCl}_3$  and refiltered. Even the second filtrates developed a cloudiness, which was more noticeable in Sample B, using petroleum benzin—alcohol extraction. The  $\text{CHCl}_3$  failed to re-dissolve some of the residue.

Much time was required to dry lipid samples to weight . . . it is difficult and time-consuming to drive off solvent in order to get a figure for total lipoids.

We question the figure for total lipoids because, by the methods used, materials other than fats are extracted. This combination of solvents will extract the theobromine and coloring matter present in the sample as well as fat.

I believe that a method for determining lecithin in chocolate products could be worked out which would shorten and simplify the procedure. Such a method would be to take the fat residue as obtained by the official method, ash, and determine  $\text{P}_2\text{O}_5$  in ash, using a colorimetric method. We are reasonably sure that the lipoids are extracted by the official method and that comparable results for lipoids can be obtained. It is then a simple matter to continue and determine phosphorus in the fat residue.

We tried this method on the samples submitted, with the following results:

	Sample A	Sample B
	per cent	per cent
Lipoids	54.3	53.1
Lipoid $\text{P}_2\text{O}_5$	0.0357	0.0573

We regret that we were unable to run check determinations because there was insufficient sample.

The exact procedure was to extract fat using the official method, evaporate, dry, and weigh. From this point, the  $\text{P}_2\text{O}_5$  in fat was determined by the procedure recommended by Smith for determining  $\text{P}_2\text{O}_5$  in fat residue of ice cream, *This Journal*, 13, 281 (1930).

S. D. Fine.—(Lipoids.) Neither a constant weight nor an increase in weight was obtained for any of the determinations after  $4\frac{1}{2}$  hours of heating. . . . The weight

used in each instance was that one after which the magnitude of the loss for a given time interval began to increase.

$P_2O_5$ .—It was necessary to place the Pt dishes containing the apparently dry alcoholic KOH treated residue in the open door of the muffle for approximately an hour before the final ashing. This was necessary to avoid excessive frothing.

M. L. Offutt.—I liked Method V(a), which extracts the fat easily and evaporates off more quickly. It appears also to give slightly higher results.

H. W. Conroy.—Petroleum benzin extraction is more rapid and apparently gives higher results.

J. H. Loughrey.—No difficulties were encountered, except for tendency of the chocolate material to carry over with the solvents; these particles of chocolate were filtered out with some difficulty. This was especially true of Method V(a), when petroleum benzin is the solvent. This is the only explanation that can be given for the discrepancy in the duplicate fat determinations.

### DISCUSSION

Most of the collaborators favor Method V(a), which seems to yield better results and is somewhat easier to follow. It has the disadvantage of yielding a turbid first extract in the case of liquor, but this may not be true with sweet chocolate.

The tentative method for lipoids under Eggs and Egg Products directs that the beaker be placed in the oven for 5–10 minutes to remove any moisture. This may not be sufficient time in the case of approximately 5 grams of chocolate lipoids. Unless the extract is thoroughly dried at this point the chloroform filtrate will be very turbid.

It is desirable to keep the funnel covered during filtration of the chloroform, as evaporation with resultant chilling condenses moisture on the insoluble brown residue, which then tends to run through the cotton. A test on this brown residue indicates that it contains a considerable amount of phosphorus, and it is believed that it should be rigidly excluded from the chloroform solution.

While some of the results quite accurately indicate the amount of added lecithin, the agreement between different collaborators on the same determination is not sufficiently close. It is hoped that the directions for Method V(a) may be amplified so as to insure close agreement among analysts.

It is recommended that work on lecithin be continued.

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## REPORT ON CHOCOLATE IN SWEET CHOCOLATE AND MILK CHOCOLATE

By W. O. WINKLER (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

No collaborative work was done this year, since the Referee considers that the work previously done on the determination of the fat-free chocolate mass in the crude fiber method warrants its adoption (with slight

modification) as a tentative method for the determination of chocolate in chocolate products containing milk solids or in chocolate products containing no milk solids.

Many data were obtained by the crude fiber procedure in arriving at the factor 0.7, which represents the ratio of the dry, fat-free cacao residue obtained after the extraction procedure used in the crude fiber method to the original dry, fat-free chocolate mass. Multiplying the weight of the dry residue obtained in the crude fiber extraction procedure by the reciprocal of the factor 0.7 (i.e., 1.43) gives the weight of the fat-free dry cacao.

For sweet chocolate, milk chocolate, skim milk chocolate, buttermilk chocolate, mixed milk chocolate, and coatings containing fat other than cacao fat the following method was used:

Extract 25-50 grams (50 grams if light color, indicating low liquor) of sample as directed in 27 and 28, *Methods of Analysis*, A.O.A.C., 1940, pp. 206-7, except to use 200 ml. of water or 1%  $\text{Na}_2\text{C}_2\text{O}_4$  solution, as the case may be, in the first aqueous extraction.

With the aid of small portions of ether (45, 20, 15 ml., etc.) transfer the residue resulting from the ether, alcohol, and aqueous extractions to a tared aluminum dish provided with a close-fitting cover. Use a small quantity of acetone and a policeman to transfer any material that sticks to the bottle. Evaporate the liquid and dry the residue in an oven at 100°C. Cover the dish, cool in a desiccator, and weigh.

To obtain the weight of fat-free dry cacao mass, multiply the weight of the residue by the factor 1.43. To obtain the weight of chocolate liquor, multiply the weight of fat-free dry cacao mass by the factor 2.062. (This factor presupposes a standard requirement of 50% fat in chocolate liquor.)

NOTE: In determining the factor 2.062, 1.5% moisture was allowed for chocolate liquor. This figure closely approximates the average moisture content of 158 samples of chocolate liquor analyzed in the 1939 survey of the Food and Drug Administration.

It is recommended\* that the method described in this report be adopted as a tentative method for the determination of the chocolate content of sweet chocolate (with or without added dairy ingredients), milk chocolate, mixed milk chocolate, skim milk chocolate, buttermilk chocolate, and coatings containing fat other than cacao fat.

## REPORT ON GUMS IN FOODS

By F. LESLIE HART (U. S. Food and Drug Administration,  
Los Angeles, Calif.), *Referee*

Referee work on detection of gums in foods during 1940 was limited to an attempt to devise a method for detection and identification of stabilizers in ice cream. This work has not yet reached the stage where a definite method can be recommended. It is being reported this year to the Association in the writer's report as Associate Referee on Ice Cream.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 24, 62 (1941).



Last year the Association adopted as tentative a method for detection of gums in mayonnaise and French dressings, *This Journal*, 22, 605 (1939). Some laboratories now report that certain spices, specifically mustard and paprika, interfere with the application of this method. Therefore, further work must be done on this method.

The methods of analysis of this Association have, since the first edition, included a tentative method for gums and dextrin in wine. This method, in fact, dates back to U.S.D.A. Bureau of Chemistry Bull. 107. Formerly commercial glucose was added to grape juice before fermentation and wines after fermentation. This glucose contained appreciable amounts of dextrin. Furthermore, glucose was added at times to grape pomace, and a pomace wine was fermented from the mixture. These practices are now obsolete in the wine industry. In California no sugar other than grape sugar may be added to wines, and in other sections, corn sugar or sucrose may be used. All of these sugars are so refined that dextrins are eliminated, and a method for detection of dextrins and gums is no longer of interest. A recommendation for its deletion has the approval of the Referee on Alcoholic Beverages.

No report was received from the Associate Referee on Gums in Starchy Foods.

#### RECOMMENDATIONS\*

It is recommended—

(1) That further study be made on the tentative method for detection of gums in soft curd cheeses published in *This Journal*, 23, 599 (1940).

(2) That the tentative method for the detection of gums and dextrins, *Methods of Analysis*, A.O.A.C., 1940, 170, 25, be deleted.

(3) That further studies be made on the tentative method for the detection of gums in mayonnaise, *This Journal*, 22, 607 (1939).

(4) That study of methods for detection of gums and stabilizers in frozen desserts and in starchy foods be continued, and extended to other foods as opportunity permits.

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No report on starchy foods was given by the associate referee.

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#### REPORT ON OILS, FATS, AND WAXES

By J. FITELSON (U. S. Food and Drug Administration,  
New York, N. Y.), *Referee*

No collaborative studies on methods were conducted during the past year, and no reports will be made by the associate referees. Some work has been done by the Associate Referee on the Refractometric Determination of Oil in Seeds in an effort to extend this method into a general procedure for oil-bearing products, but no report will be made at this time.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 58 (1941).

Recent articles on the thiocyanogen number<sup>1,2,3,4</sup> indicate that the observed thiocyanogen values for linoleic and linolenic acids are not in accord with the theoretical values used in the calculations in the present tentative method. This difference is particularly serious in fats containing appreciable quantities of linolenic acid, but for most of the edible fats the effect on the calculated proportions of acids is small. Hilditch and Murti<sup>4</sup> recommend that these theoretical values be replaced by agreed empirical values when general agreement has been reached as to the precise values and the conditions of determination. In view of the uncertain status of the data obtained by this method for fats containing high proportions of linoleic and linolenic acids, it is recommended that further collaborative studies on the thiocyanogen number for fats and oils be temporarily discontinued.

Methods for the determination of olive oil in admixture with other oils are being investigated by the Referee. A study was made of the method proposed by Grossfeld and Timm,<sup>5</sup> based on a modified iodine number of the unsaponifiable matter, and this method was shown to be erratic and unreliable. The high iodine value of the unsaponifiable matter of olive oil is due largely to the presence of an unsaturated hydrocarbon, squalene. Although some work has recently been done on the chemical behavior of squalene by Taufel, Thaler, and Widman,<sup>6</sup> methods for the isolation and determination of this compound are still in the formative stage. Studies on the characteristics of squalene and its compounds are being made by the Referee.

The Fat Analysis Committee of the American Chemical Society and the American Oil Chemists' Society has recently published the results of its investigations of several methods.<sup>7</sup> With the exception of the titer test, their recommended methods show only slight variations from the present A.O.A.C. methods. The important modifications in the F.A.C. titer method are vertical stirring in place of horizontal stirring, detailed specifications for the apparatus, and the use of a partial immersion thermometer in place of the total immersion. The Referee has begun a study of this F.A.C. modification and has obtained results by the modified method that have been much more uniform and consistent than those obtained by the A.O.A.C. method. Since these modifications involve no fundamental changes in the titer test, the Referee believes that a collaborative study is not necessary. Examination of this method will be continued with the view to the adoption of those modifications that will make the present A.O.A.C. method more precise.

The present official method for unsaponifiable matter, while satisfactory

<sup>1</sup> Riemenschneider and Wheeler, *Oil and Soap*, 16, 219 (1939).

<sup>2</sup> Kass, Lundberg, and Burr, *Ibid.*, 17, 50 (1940).

<sup>3</sup> Kass, Loeb, Norris, and Burr, *Ibid.*, 118.

<sup>4</sup> Hilditch and Murti, *Analyst*, 65, 437 (1940).

<sup>5</sup> Z. *Untersuch. Lebensmittel.*, 77, 249 (1939).

<sup>6</sup> *Biochem. Z.*, 300, 354 (1939).

<sup>7</sup> *Oil and Soap*, 17, 127 (1940); *Ind. Eng. Chem., Anal. Ed.*, 12, 379 (1940).

for most of the edible oils, can not be used for oils, such as fish liver oils, which contain large quantities of unsaponifiable matter. The last A.O.A.C. collaborative study of methods for unsaponifiable matter was made in 1926, and at that time it was felt that it was inadvisable to have more than one method for the same determination, except under special conditions, *This Journal*, 9, 81, 247 (1926). Since that time, many new fats have come into use and several new methods have been proposed or adopted by various organizations. The Referee recommends that a study of methods for unsaponifiable matter be made. The most satisfactory method should be applicable to all oils or fats, or, failing that, should be of widest application with necessary modifications for special use. The Fat Analysis Committee, in its recent report, announced its program for the study of several methods of analysis, including methods for unsaponifiable matter. In order to insure as much uniformity as possible in the methods selected, arrangements are being made for closer cooperation with this committee.

#### RECOMMENDATIONS\*

It is recommended—

(1) That collaborative studies of the modified Kaufmann method for thiocyanogen number be temporarily discontinued.

(2) That studies on the application of the method for refractometric determination of oils in flaxseed to other oil seeds and oil-bearing materials be continued.

(3) That studies on the Polenske method be continued.

(4) That studies on the development of methods for the determination of olive oil in admixture with other oils be continued.

(5) That the modifications of the titer test approved by the Fat Analysis Committee of the A.C.S.—A.O.C.S. be studied.

(6) That a collaborative study be made of methods for the determination of unsaponifiable matter.

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No report on refractometric determination of oil in seeds was given by the associate referee.

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No report on thiocyanogen number was given by the associate referee.

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No report on Polenske number was given by the associate referee.

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No report on coffee and tea was given by the associate referee.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 59 (1941).

## REPORT ON MICROBIOLOGICAL METHODS

By ALBERT C. HUNTER (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

Last year progress in the development of microbiological methods for the examination of foods was marked by the presentation of recommendations for tentative adoption of methods for the examination of canned vegetables, sugar, and frozen eggs. Since the last annual meeting the Associate Referee on Eggs and Egg Products has organized certain collaborative studies, the results of which are being reported at this meeting. The Referee concurs in the recommendations presented by the associate referee. With the foundation that has been laid it would probably be in order to move now for action toward the final adoption of the methods for canned vegetables, sugar, and frozen eggs, but in order to allow time to iron out a few wrinkles in those methods and also to permit further development of other methods in the canned foods series originally taken within the scope of the program the Referee prefers to recommend no further action on the three groups of methods named other than the appointment of associate referees to continue guidance of the necessary programs of study. Under the circumstances, there will be no report on canned vegetables and sugar this year.

There has not been the desired and expected progress in the handling of work on methods for the examination of canned fish products and canned meats. Before these methods may be presented for tentative adoption there are probably some minor alterations to be made in the directions previously reported. There is every reason to conclude that those changes can be made with little difficulty and that during the coming year the associate referees can prosecute appropriate collaborative studies to bring progress on fish and meats into line with that on the other commodities under consideration in the program.

The work under Canned Fruits has actually been restricted to methods for the examination of canned tomatoes and tomato products since the primary purpose of the studies is to develop methods for the microbiological examination of foods definitely in the acid range, and of such products tomatoes are of greatest importance from the viewpoint of possible microbial spoilage. A method that appears to be satisfactory has been formulated and is being presented for tentative adoption at this meeting. Collaborative studies now under way will be continued. The recommendations of the associate referee are herewith endorsed.

At the present time, then, there are as tentative methods procedures for the examination of canned vegetables, canned tomato products, sugar, and frozen eggs with every prospect of the early development of methods for canned fish and canned meats. It seems in order to push ahead by broadening the field of operations. Of late, considerable attention has been

given to the bacteriological examination of shelled nuts, and the matter becomes of sufficient importance to warrant development of approved methods. Also, the growth of the frozen fruit and vegetable industry and the need for recognized methods for the examination of its output calls for attention to this class of commodities.

Therefore it is the recommendation\* of the Referee that associate referees again be appointed for (a) Canned Vegetables, (b) Canned Fruits (Tomato Products), (c) Canned Fish Products, (d) Canned Meats, (e) Sugar, and (f) Eggs and Egg Products, and, in addition, for (g) Frozen Fruits and Vegetables and (h) Nuts and Nut Products.

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No report on canned fish products was given by the associate referee.

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No report on canned meats was given by the associate referee.

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No report on canned vegetables was given by the associate referee.

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### REPORT ON MICROBIOLOGICAL METHODS FOR THE EXAMINATION OF CANNED FRUITS, TOMATOES, AND OTHER ACID FOODS

By B. A. LINDEN (Division of Bacteriology, U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Since the last report on acid canned foods, *This Journal*, 21, 454 (1938), investigational and collaborative work has been carried on, and a large number of specimens of acid canned foods have been examined by this method.

The medium used for the detection of aciduric spoilage organisms has been given additional study and has been modified. The most important change in the medium involves the adjustment of the reaction to constant reaction pH 4.5 by use of buffers instead of addition of lactic acid. The addition of 11 grams of citric acid, plus 12 grams of potassium citrate (monohydrate) per liter of bouillon has been found satisfactory for this purpose.

This method and the modified medium have been found particularly useful in the detection of certain aciduric anaerobic organisms that have been encountered in large-scale spoilage of packs of canned tomatoes and canned pineapple. The method has also shown its usefulness for the detection of packs of canned tomatoes that have been processed in open kettle at comparatively low temperatures but otherwise sound because of the absence of aciduric spoilage bacteria.

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 59 (1941).

It is recommended\* that this method be adopted as a tentative method and that collaborative studies be continued.

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No report on sugar was given by the associate referee.

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The Associate Referee on Microbiological Methods for Examination of frozen Egg Products will not present his report this year because of delay in assembling the collaborative results. The revisions that were made in the methods were published in *This Journal*, 24, 59, 93 (1941).

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#### ERRATA AND EMENDATIONS

##### *Methods of Analysis, A.O.A.C., 1940*

Page	Section	
36	54, par. 2, line 3	Change " $H_2PO_4$ " to " $H_3PO_4$ ."
37	58	Change last three lines to read as follows: "Continue as directed in XXVII, 60, beginning, 'Add 0.3 g of KIO <sub>4</sub> for each 15 mg. of Mn present'."
369	60, line 7	Place period after $H_2O$ and change word "and" to "Add."
706	19, Col. 8, last line	Change "100.00" to "00 00."

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 24, 59 (1941).

## CONTRIBUTED PAPERS

### SEPARATION AND ESTIMATION OF MERCURY, BISMUTH, AND ZINC IN "SKIN BLEACHES"

By GEORGE McCLELLAN (Cosmetic Division, U. S. Food and Drug Administration, Federal Security Agency, Baltimore, Md.)

Mercury, bismuth, and zinc, usually mixed with petrolatum or lanolin to form a salve, are present in certain types of "skin bleaches."

Numerous procedures for separating mercury and bismuth have been published, but selective precipitation of mercury as the sulfide apparently has not been extensively investigated. In the experiments to be described, a clean-cut separation of mercury from bismuth was effected by precipitation of mercury as the sulfide from (1+1) hydrochloric acid. There appears to be no co-precipitation of bismuth from this medium.

If the filtrate from the mercury determination is diluted with an equal volume of water, bismuth apparently can be quantitatively precipitated by the addition of more hydrogen sulfide. If mercury alone is present with zinc, it should be precipitated from (1+4) or stronger hydrochloric acid. In media of lesser acidity, co-precipitation of zinc may occur (see Tables 2 and 3).

#### EXPERIMENTAL

A mixture containing known quantities of petrolatum, ammoniated mercury, bismuth subnitrate, and zinc oxide was analyzed in duplicate according to the following procedures:

*A. Chloroform-soluble material (petrolatum).*—Weigh ca. 2.5 grams of the mixture into a 300 ml. separatory funnel. Add 100 ml. of (1+1) HCl, and extract with successive portions of 75, 25, and 25 ml. of  $\text{CHCl}_3$ . Filter the combined  $\text{CHCl}_3$  extracts through absorbent cotton into a tared dish. Heat on the steam bath until the  $\text{CHCl}_3$  has evaporated and for 15 minutes thereafter. Cool, and weigh.

*B. Mercury.*—Drain the acid extract into a 250 ml. volumetric flask. Wash the funnel with (1+1) HCl, adding the washings to the flask. Make to volume with the HCl. Shake well, and transfer a 100 ml. aliquot sample to a 500 ml. wide-mouthed Erlenmeyer flask. Insert a stopper fitted with an inlet and an outlet tube, having the inlet tube extend only to a point ca.  $\frac{1}{4}$  inch above the surface of the liquid. Run in  $\text{H}_2\text{S}$  for 5 minutes, swirling the flask occasionally. (The precipitated  $\text{HgS}$  is yellow, but will turn black if allowed to stand in the light for 15 minutes. The color change does not alter the composition, however.) Filter through a tared Gooch crucible, and wash with (1+1) HCl. Transfer the filtrate to the flask in which precipitation was made. Wash the residue with alcohol and ether, extract for 15 minutes with  $\text{CS}_2$  in a Bailey-Walker extraction apparatus, and again wash with alcohol and ether. Dry for 15 minutes at 100° C. Weigh as  $\text{HgS}$ .

*C. Bismuth.*—Dilute the filtrate from *B* with 1.5 times its volume of water. (In the experiments summarized in Table 1 an equal volume of water was used, so that the acid was approximately 3 *N*. The extra volume specified here can do no harm,

however, and may provide an extra margin of safety.) Pass in a rapid stream of  $H_2S$ . (After 3 or 4 minutes  $Bi_2S_3$  begins to come down.) Continue to pass in the gas for 5 minutes after precipitation has become copious. Filter through a tared Gooch crucible, wash first with (1+9)  $HCl$  saturated with  $H_2S$ , and then with alcohol and ether. Continue as directed for  $HgS$  in *B*. Weigh the residue as  $Bi_2S_3$ .

*D. Zinc.*—The filtrate from *C* is often too large for convenient handling.

Take a fresh 100 ml. aliquot of the solution prepared in *B*, dilute it with 1.5 times its volume of water, and precipitate mercury and bismuth together according to the method for bismuth in *C*. Filter through a Gooch crucible, wash with (1+9)  $HCl$  saturated with  $H_2S$ , and discard the precipitate. Transfer filtrate and washings to a 500 ml. Erlenmeyer flask, boil free of  $H_2S$ , and neutralize with  $NH_4OH$ . Determine zinc by the phosphate method.<sup>1</sup>

## RESULTS

TABLE 1

PRESENT	NO. 1		NO. 2	
	FOUND	RECOVERY	FOUND	RECOVERY
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
$CHCl_3$ -sol. material (petrolatum)	60.00	59.95	60.00	100.00
$Hg$ as $HgNH_2Cl$	10.00	10.01	9.93	99.3
$Bi$ as bismuth sub-nitrate	10.00	9.81	9.95	99.5
$ZnO$	20.00	20.04	19.93	99.7
Total	100.00	99.81	99.81	

Two mixtures containing known amounts of petrolatum, mercury, and zinc were analyzed in duplicate. The results in Table 2 were obtained by precipitating mercury as the sulfide from (1+19) hydrochloric acid, and those in Table 3 by conducting the precipitation in (1+4) hydrochloric acid.

TABLE 2

PRESENT	NO. 1		NO. 2	
	FOUND	RECOVERY	FOUND	RECOVERY
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
$CHCl_3$ -sol. material (petrolatum)	81.80	81.82	81.94	100.2
$ZnO$	13.20	12.90	12.91	97.8
$Hg$ as $HgCl_2$	5.00	5.38	5.31	106.3
Total	100.00	100.10	100.16	

<sup>1</sup> Scott, Standard Methods of Chemical Analysis, 5th ed., p. 1058.



TABLE 3

PRESENT	NO. 1		NO. 2	
	FOUND	RECOVERY	FOUND	RECOVERY
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
CHCl <sub>3</sub> -sol. material (petrolatum)	81.80	82.00	81.95	100.2
ZnO	13.20	13.16	13.20	100.0
Hg as HgCl <sub>2</sub>	5.00	5.05	4.95	99.1
Total	100.00	100.21	100.10	

## CONCLUSIONS

(1) Mercury and bismuth may be separated by precipitation of mercury as the sulfide in (1+1) hydrochloric acid.

(2) Either bismuth alone or bismuth and mercury together are quantitatively precipitated by hydrogen sulfide in (1+3) hydrochloric acid.

(3) Mercury is quantitatively separated from zinc when precipitated as the sulfide in (1+4) hydrochloric acid, but in (1+19) hydrochloric acid appreciable amounts of zinc are co-precipitated.

## SUMMARY

A method has been presented for the successive separation of mercury, bismuth, and zinc in mixtures containing salts of these three metals. Precipitation with hydrogen sulfide at different acidities is utilized for the separation of the elements. Typical results are given.

## ADDITIONAL REFERENCES

- A. STAHLER, *Chem. Ztg.*, **31**, 615-6, through *Chem. Abs.*, **2**, 242 (1908).  
 A. P. CASTANARES, *Orig. Com. 8th Intern. Congr. Appl. Chem. (appendix)*, **25**, 39-40, through *Chem. Abs.*, **7**, 2171 (1913).  
 E. ZINTL and G. REINACKER, *Z. anorg. allgem. Chem.*, **161**, 385-92, through *Chem. Abs.*, **21**, 3173 (1927).  
 SCOTT, *Standard Methods of Chemical Analysis*, 5th ed., pp. 576 and 1058.  
 LUNDELL and HOFFMAN, *Outlines of Methods of Chemical Analysis*, pp. 49-54.  
 D. C. GARRATT, *Drugs and Galenicals*, pp. 49-55 and 201-214.

## RELATION OF CHEMICAL ANALYSES OF BUTTER TO ITS VITAMIN A POTENCY\*

By G. S. FRAPS, A. R. KEMMERER, and W. W. MEINKE (Agricultural Experiment Station, College Station, Texas)

Although the spectrographic method for the determination of vitamin A and carotene in butter is quite commonly used, little work has been done on the relation of the quantity of spectro vitamin A and carotene to the vitamin A potency measured by biological means. Baumann and Steenbock (1) compared results obtained spectroscopically with results obtained biologically on different samples of butter believed to be similar and estimated that 20 micrograms of vitamin A was equivalent to 66 biological units or 85 International Units. From the analyses of 32 samples of butter Fraps and Kemmerer (2) developed two equations to show the relation between the vitamin A and carotene found spectroscopically and the number of Sherman-Munsell rat units. Leuschen and coworkers (3), from 9 samples of butters, reported that one microgram of spectro vitamin A was equivalent to approximately 1.56 International Units and 1 microgram of carotene to approximately 1.65 International Units. Kunerth and coworkers (4), in studying the effect of drought on the vitamin A content of butter fat, used the method of Leuschen *et al.* to calculate the vitamin A and carotene found spectroscopically into International Units. Dornbush, Peterson and Olson (5), in a study of the vitamin A potency of market milks, calculated the vitamin A potency from the spectrographic analysis by dividing the micrograms of carotene by 0.6 and the micrograms of vitamin A by 0.3 and adding the results.

## METHOD OF PROCEDURE

The samples of butter used were furnished by O. C. Copeland of the Division of Dairy Husbandry. The spectro vitamin A content of the butters was determined by the method published in Bulletin 560 of the Texas Agricultural Experiment Station (2). In all the work the extinction coefficient used for vitamin A was 1600, which was also used in previous work and by Dornbush, Peterson and Olson (5), although recent investigations (6) indicate that the extinction coefficient is higher.

The carotene was estimated with the aid of a Bausch and Lomb spectrophotometer. Five grams of the melted fat was placed in a 10 ml. volumetric flask and diluted to volume with light petroleum benzin. This mixture was placed in the cup of the spectrophotometer, and the density at 470 and 480  $m\mu$  was read against petroleum benzin. The amount of carotene was calculated by multiplying the density by the corresponding factors in Table 1. These factors are for a 2 cm. depth of solution; for a 1 cm. depth the density was multiplied by 2, and for a 4 cm. depth, divided by 2.

\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D.C., October 28-30, 1940.

The factors in Table 1 were devised as follows: Crystalline carotene manufactured by the S.M.A. Corporation was purified according to a method published by Treichler, Grimes, and Fraps (7); 20 mg. of the purified carotene was dissolved in 1 ml. of chloroform and diluted to 50 ml. with light petroleum benzin and 2 ml. of this solution was made up to 100 ml. with petroleum benzin. This constituted the stock solutions. Desired quantities of the stock solution were placed in 10 ml. volumetric flasks, 5 grams of a very light colored melted butter fat was added, and the mixture was diluted to volume with petroleum benzin. The density of absorption of these solutions was determined at 470 and 480  $m\mu$  against a blank containing the same proportion of petroleum benzin and butter fat.

TABLE 1.—*Factors for conversion of color density for a 2 cm. depth to p.p.m. of carotene in butter*

		FACTOR
For density of absorption at 480 $m\mu$		
	0.00–0.33	4.86
	0.34–0.52	4.62
	0.53–0.66	4.85
	0.67–0.82	4.88
	0.83–1.00	4.80
For density of absorption at 470 $m\mu$		
	0.00–0.31	5.16
	0.32–0.50	4.80
	0.51–0.65	4.92
	0.66–0.80	5.00
	0.81–0.98	4.90

The vitamin A potency of the butter fats was estimated by the U.S.P. method used in this laboratory (8), modified, in that standards of pure carotene in Wesson oil were used instead of the U.S.P. reference oil. White rats were used. The standard carotene was fed at three levels, and the butter fat under test at two levels.

## RESULTS

In Table 2 are given the spectro vitamin A, the carotene contents, and the biological potency in International Units of 29 samples of butter fat. One microgram of carotene in the butters is assumed to have a value of 1.7 International Units (9), though there is some evidence (10) that 6 per cent of the supposed carotene may be xanthophyl and that carotene in butter may have a higher vitamin A potency than carotene in oil (11). When the vitamin A potency ascribed to the carotene is subtracted from the vitamin potency as determined by biological methods, the remainder is due to vitamin A. When the International Units due to vitamin A are divided by the parts per million of spectro vitamin A, the value of 1

microgram of spectro vitamin A is found to range from 1.0 to 7.4 International Units, with an average of 3.2 units for the 29 butters.

In Bulletin 560, referred to previously, the equation:

$$I_u = 8.16 (S - 0.8) + 1.68C,$$

was used to convert spectro vitamin A and carotene into International Units. In this equation  $S$  is the parts per million of spectro vitamin A,  $C$  the parts per million of carotene and  $I_u$  the number of International Units. This equation worked well for the 32 samples of butter fat tested by the Sherman-Munsell method and reported in Bulletin 560 (2) but when it was applied to the butters tested by the modified U.S.P. method (Table 2) the calculated International Units were much higher than were those obtained by biological analyses.

The vitamin A potency of the butter fat can then be calculated by the equation:  $I_u = 3.2S + 1.7C$ , in which  $I_u$  is International Units per gram,  $S$  is spectro vitamin A, and  $C$  is carotene in parts per million. Values so calculated are given in Table 2. The average of the differences is 4.1, and the standard deviation is 6.2.

A somewhat better equation can be developed in consideration of the fact that all of the absorption of light at 328  $m\mu$ . is not due to vitamin A, and the presence of such substances (called pseudo vitamin A) makes the calculation too high for butters low in vitamin A and too low for those high in vitamin A, as can readily be seen in Table 2.

The equation developed is:

$$I_u = (S - 0.5)4 + 1.7C.$$

The average difference of the calculated biological values from those found is 3.9, and the standard deviation is 5.5. If one sample is omitted, the average difference is 3.2 and the standard deviation is 3.7. The second equation, therefore, is better.

#### DISCUSSION

From the second equation developed in this work the value of 1 microgram of vitamin A in butter fat was found to be equal to 4 International Units. This figure is obtained when the 16 butters in Table 2, which have a value of 3.1–5.5 International Units per microgram of spectro vitamin A, are averaged. If the values for the 28 butters (except 52108, which is excluded) are averaged and the presence of pseudo vitamin A is disregarded, the average is 3.2. The latter value is nearly the same as the 3.3 found by Holmes and Corbet (6) for crystalline vitamin A. However, when 2100 is used for the extinction coefficient of pure vitamin A, which is nearer the correct value than 1600, the value for 1 microgram of vitamin A is 2.4 International Units. Moll and Reid (12) report the factor to convert  $E_{1\%}^{1\text{cm}}$ , 328  $m\mu$  in fish oil to International Units to be 1800–1900

when saponified and 3300–3700 when not saponified. According to these results, 1 microgram of vitamin A is equal to 2.9–3.0 International Units for saponified material and to 5.3–5.9 units for the untreated material. Grab (13) confirms the results of Moll and Reid (12). The higher potency of the unsaponified material is due to the presence of an ester of vitamin

TABLE 2.—*Vitamin A potency in International Units, spectro vitamin A, and carotene in butter fat*

LABORATORY NUMBER	CAROTENE	SPECTRO VITAMIN A	VITAMIN A POTENCY	VITAMIN A POTENCY EQUAL TO 1 MICROGRAM OF SPECTRO VITAMIN A	VITAMIN A POTENCY CALCULATED EQUATION A	VITAMIN A POTENCY CALCULATED EQUATION B
	p.p.m.	p.p.m.	International Units/gram	units	International Units	International Units
51206	1.8	1.6	5	1.2	8	8
51214	2.9	2.1	7	1.0	12	11
51876	1.0	1.4	6	3.1	6	5
51673	1.3	1.6	6	2.4	7	7
51670	2.2	2.6	8	1.7	12	12
52008	1.7	1.7	5	1.2	8	8
52009	0.9	2.0	5	1.8	8	8
52225	1.3	1.6	6	2.4	7	7
Average (8)	1.6	1.8	6	1.9	9	8
51212	2.8	2.8	16	4.0	14	14
51454	1.4	3.9	12	2.5	15	16
51455	1.1	2.5	13	4.4	10	10
51460	5.6	3.9	19	2.4	22	23
51875	1.2	2.2	10	3.6	9	9
51679	3.2	3.5	18	3.6	17	17
Average (6)	2.6	3.1	15	3.4	15	15
51204	3.1	4.0	23	4.4	18	19
51675	3.6	4.3	25	4.4	20	21
52110	10.9	4.3	29	2.4	32	34
52107	16.0	6.6	53	3.9	48	51
52112	11.1	6.1	36	2.8	38	41
52109	9.4	6.1	35	3.1	35	38
52104	7.4	4.8	34	2.4	31	33
52541	5.6	5.4	27	3.2	27	29
52542	4.3	3.8	23	4.1	20	21
52545	4.3	4.1	21	3.3	20	25
52547	5.1	4.6	31	4.8	23	25
52108	11.4	5.9	63	7.4	38	41
53049	4.2	4.4	29	4.0	21	23
53051	4.8	5.4	31	4.2	26	28
53540	4.0	4.2	30	5.5	20	22
Average (15)	7.0	4.9	33	4.1	28	30

A, which is more potent than alcohol produced by saponification. The value of 4.0 International Units for 1 microgram of vitamin A in butter appears to be nearer the true potency than the value 3.2, which is not corrected for pseudo vitamin A. This value of 4.0 becomes 3.1 International Units when 2100 is used as the extinction coefficient. Butters may very possibly contain esters of vitamin A that are more potent than the vitamin A.

The value of 4.0 for vitamin A in the work presented here is much less than the value of 8.0 previously published (2). This difference may be attributed partly to the difference in the biological method used and partly to the differences in the sources of butter. The value of 4.0 seems to agree better with other published values for vitamin A than does the previous value.

#### SUMMARY

(1) From the analyses of 29 samples of butter fat for carotene, spectro vitamin A, and vitamin A potency in International Units, it was found that the biological potency in International Units could be calculated from the analyses by the equation  $I_u = S3.2 + 1.7C$ , or  $I_u = (S - 0.5)4 + 1.7C$ .  $I_u$  is the number of International Units per gram,  $S$  the spectro vitamin A in parts per million, and  $C$  the carotene in parts per million. The second equation gave better results.

(2) With 23 of the 29 samples of butter analyzed, the International Units per gram calculated by the second equation differed from the number of International Units found by 4 units or less. Five of the samples differed by 5 to 8 units and one by 22 units.

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## ESTIMATION OF BENZOCAINE AND ITS SEPARATION FROM ACETANILID

By EUGENE H. WELLS (U. S. Food and Drug Administration,  
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A method for the separation of benzocaine (ethyl *p*-aminobenzoate) from acetanilid has been developed by Bruening.<sup>1</sup> It is based on the extraction of the acetanilid from a (1+1) hydrochloric acid solution of the mixture with chloroform, no appreciable amount of benzocaine being extracted from acid of that strength. The benzocaine is extracted from ammoniacal solution with chloroform and determined gravimetrically after the residue has been heated in an oven at 100° C. for 8 minutes. In view of the volatility of benzocaine at elevated temperatures, a volumetric method for its estimation seems preferable.

Leulier and Dinet<sup>2</sup> brominated benzocaine, using hydrogen bromide and hydrogen peroxide, but made no attempt to apply the method quantitatively. Fiyalkov and Yamposka<sup>3</sup> estimated benzocaine by brominating in acid solution with an excess of 0.1 *N* potassium bromate-bromide solution. An excess of 0.1 *N* arsenous oxide solution and a small quantity of methyl red indicator were added, and the excess arsenous oxide was titrated with 0.1 *N* potassium bromate solution to the disappearance of the red color of the dye. This method was also studied by Hoffmann.<sup>4</sup> Valencien and Deshusses<sup>5</sup> successfully determined benzocaine by bromination in an acid solution with a measured excess of standard bromate-bromide solution. After the reaction mixture had stood an hour in the cold, an excess of potassium iodide solution was added and the free iodine was titrated with standard thiosulfate solution, starch solution being used as an indicator. As this method seemed most feasible, its application to this problem was studied.

## EXPERIMENTAL

Twenty-five grams of benzocaine of commercial quality was dissolved in 100 ml. of CHCl<sub>3</sub>, and the solution was shaken with a small quantity of dilute ammonia water, washed with water, filtered, and evaporated under an air blast at room temperature. The residue was dissolved in 100 ml. of hot 95% alcohol, and hot water (ca. 70° C.) was added until near saturation. The precipitate obtained on cooling was dried over H<sub>2</sub>SO<sub>4</sub> under reduced pressure and had a melting point of 87.8°–90.6° C., uncorrected.

A sample of benzocaine was weighed into an iodine flask and dissolved with 10 ml. of HCl and about 200 ml. of water; 0.1 *N* bromine solution was added from a buret until a slight excess was evidenced by the light yellow color. The flask was stoppered, shaken, and allowed to stand for 5 minutes. An excess of KI solution was added, loss of any bromine being avoided, and the liberated iodine was titrated with

<sup>1</sup> Private communication of Charles F. Bruening, Baltimore Station, U. S. Food and Drug Administration.

<sup>2</sup> *J. pharm. chim.*, 8, 57 (1928).

<sup>3</sup> *Arch. Pharm.*, 270, 203 (1932), and *Farm. Zhur.*, 13–16 (1932).

<sup>4</sup> *Apoth-Ztg.*, 47, 686 (1932).

<sup>5</sup> *Mitt. Lebensm. Hyg.*, 30, 246 (1939).

the  $\text{Na}_2\text{S}_2\text{O}_3$  solution, starch solution being used as an indicator. The average recovery of three assays was 99.9%.

Pure acetanilid was prepared from aniline and acetic anhydride, decolorized, recrystallized three times from hot water, and dried in an oven at  $60^\circ\text{C}$ ., melting point  $113^\circ\text{--}116^\circ\text{C}$ . uncorrected. The acetanilid was analyzed by the A.O.A.C. method of hydrolysis and titration with 0.1 *N* bromine solution. The results of four assays varied from 98.3 to 100.7%, with an average of 99.9%.

An attempt was made to apply Bruening's method to a mixture containing 2.5 per cent of benzocaine and 20.0 per cent of acetanilid by the use of the bromometric procedure for the estimation of benzocaine. Recoveries of benzocaine ranged from 158 to 244 per cent, while those of acetanilid ranged from 84 to 94 per cent. Modification of the procedure slightly improved the results but indicated that all the acetanilid could not be extracted from (1+1) hydrochloric acid by chloroform, probably due to hydrolysis of the acetanilid.

Since acetanilid is approximately 4.5 times more soluble in (1+1) hydrochloric acid than in 6 *N* sulfuric acid, the distribution ratios of benzocaine and acetanilid between these acids and chloroform and ether were studied. The results indicate that chloroform and 6 *N* sulfuric acid are a better combination. Using 6 *N* sulfuric acid and chloroform, the writer studied the effect of varying the number of extractions and the volume of the immiscible solvent for both acetanilid and benzocaine. It was found that the best separation could be obtained by dissolving the mixture in 50 ml. of chloroform and passing this chloroform solution successively through three separatory funnels containing 30, 20, and 10 ml. portions of 6 *N* sulfuric acid and one funnel containing 10 ml. of water. Four 20 ml. portions of chloroform were subsequently passed through the same series of funnels and all the chloroform extracts were combined. When water-soluble excipients, such as sucrose, were present, they were removed by two washings with 20-40 ml. and 10 ml. portions of water before the chloroform solution was passed through the sulfuric acid.

The method follows:

#### REAGENTS

*Sulfuric acid*.—6 *N*. 169 ml. (or 310 grams) of 95%  $\text{H}_2\text{SO}_4$ , sp. gr. 1.84, diluted to 1 liter.

*Sodium thiosulfate solution*.—0.1 *N*. Made according to Kassner & Kassner<sup>6</sup> by dissolving 24.8 grams of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1 liter of cold sterile water and adding 0.4 ml. of  $\text{CHCl}_3$  and 0.01 gram of  $\text{Na}_2\text{CO}_3$  per liter. The solution was stored in a rubber-stoppered brown bottle and standardized with pure  $\text{I}_2$  or  $\text{KIO}_3$ .

*Potassium bromide-bromate solution*.—0.1 *N*. 3.0 grams of  $\text{KBrO}_3$  and 25 grams of  $\text{KBr}$  dissolved in distilled water, diluted to 1 liter, and standardized against the standard thiosulfate solution.

*Potassium iodide solution*.—Either 10% solution was used or the required quantity of solid  $\text{KI}$  was placed in the cup of the  $\text{I}_2$  flask and dissolved in a small quantity of water.

<sup>6</sup> *J. Ind Eng Chem, Anal Ed*, 12, 655 (1940).



TABLE 1.—Separation and estimation of benzocaine and acetanilid (%)

SAMPLE NO.	ANALYST	BENZOCAINE			ACETANILID		
		PRESENT	FOUND	RECOVERY	PRESENT	FOUND	RECOVERY
1	EHW	2.50	2.49	99.7	20.00	20.07*	100.3
1	EHW	2.50	2.55	101.9	20.00	19.90†	99.5
1	EHW	2.50	2.52	101.0	20.00	19.84†	99.2
1	CDW	2.50	2.51	100.4	20.00	20.03	100.1
1	CDW	2.50	—	—	—	—	—
1	LHW	2.50	2.61	104.4	20.00	19.8	99.0
2	EHW	1.25	1.27	101.8	20.00	19.96†	99.8
2	EHW	1.25	1.29	103.2	20.00	19.93†	99.7
2	EHW	1.25	1.28	102.4	20.00	19.79	98.9
2	CDW	1.25	1.25	100.0	20.00	19.96 (20.16)*	99.8
2	CDW	1.25	1.30	104.0	20.00	19.91 (20.05)*	99.5
2	LHW	1.25	1.35	108.0	20.00	19.8	99.0
3	EHW	2.50	2.52	100.9	20.00	19.96	99.8
3	EHW	2.50	2.51	100.5	20.00	20.03*	100.2
3	DCG	2.50	2.51	100.5	20.00	19.90	99.5
3	DCG	2.50	2.51	100.5	20.00	19.83	99.15
4	EHW	5.00	4.91	98.3	10.00	9.97	99.7
4	EHW	5.00	4.97	99.4	10.00	9.98	99.8
4	DCG	5.00	4.91	98.2	10.00	9.96	99.4
4	DCG	5.00	4.92	98.4	10.00	9.95	99.5

\* Determined gravimetrically.

† Acetanilid determined by method of Kethley.

**Starch indicator.**—A 2% solution was made by dissolving 4 grams of soluble starch in 200 ml. of boiling water. After boiling a short time, the solution was cooled and placed in a bottle with a small globule of mercury.

## PROCEDURE

A weighed portion of the powdered and mixed sample, sufficient to yield approximately 0.1 gram of benzocaine, was placed in a 120 ml. separatory funnel and shaken with 50 ml. of  $\text{CHCl}_3$  and 20–40 ml. of water, depending on the quantity of soluble excipient present. The  $\text{CHCl}_3$  solution was drawn into another separator and washed with 10 ml. of water. It was then filtered through a cotton pledget into a separator containing 30 ml. of the  $\text{H}_2\text{SO}_4$  at room temperature and shaken vigorously. The  $\text{CHCl}_3$  solution was then passed through a system of three separators containing 20 and 10 ml. of the  $\text{H}_2\text{SO}_4$  and 10 ml. of water, respectively. The  $\text{CHCl}_3$  solution was filtered from the last separator through a cotton pledget into a beaker or volumetric flask. Four 20 ml. portions of  $\text{CHCl}_3$  were passed through the entire system of separators, and the combined  $\text{CHCl}_3$  extracts (or aliquot) were evaporated nearly to dryness with a blast of air on the steam bath. The acetanilid was hydrolyzed either by the A.O.A.C. method, *Methods of Analysis*, A.O.A.C., 1940, 561, 4(b), beginning "Add 10 ml. of  $\text{H}_2\text{SO}_4$  (1+9)," or with 4 N HCl according to the procedure of Kethley,<sup>7</sup> the latter procedure effecting a considerable saving in time. The hydro-

<sup>7</sup> *This Journal*, 23, 782 (1940).

lyzed acetanilid was titrated according to the A.O.A.C. method,<sup>2</sup> the 0.1 *N* bromide-bromate solution being used. When the quantity of acetanilid was large, an aliquot was taken so as to give 0.1–0.2 gram of acetanilid for titration.

The  $H_2SO_4$  solutions and the wash water in the last separator were transferred to a 500 ml.  $I_2$  flask, and sufficient water was added to give a volume of about 200 ml. An excess of 0.1 *N* bromine solution was added, and the stoppered flask was shaken vigorously. An excess of the KI solution was added, and the free iodine was titrated with the thiosulfate solution, starch being used as an indicator. The flask was shaken vigorously near the end of the titration.

The acetanilid was not permitted to stand in contact with the acid solution any longer than necessary as hydrolysis takes place and the resulting aniline is determined with the benzocaine.

1 ml. of 0.1 *N*  $Br_2$  solution = 0.004127 gram of benzocaine.

1 ml. of 0.1 *N*  $Br_2$  solution = 0.002252 gram of acetanilid.

### RESULTS

Four mixtures of benzocaine, acetanilid, and sugar were prepared and analyzed by several chemists by the above method. The results are given in Table 1.

### SUMMARY

(1) The bromometric method of Valencien and Deshusses for the estimation of benzocaine was studied, and with slight modification it was found to be suitable for the volumetric estimation of benzocaine.

(2) The adaptation of the volumetric procedure to a modification of the Bruening method for the separation of mixtures of benzocaine and acetanilid was also studied.

(3) Mixtures of benzocaine, acetanilid, and sugar were studied collaboratively, and the results are submitted.

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## DETERMINATION OF PURE CAROTENE IN GREEN VEGETABLES AND GREEN FEEDS

By G. S. FRAPS, W. W. MEINKE, and A. R. KEMMERER (Agricultural Experiment Station, College Station, Texas)

The methods for the determination of pure carotene (1) have been applied chiefly to alfalfa products and other dried feeds. In fresh green vegetables and green feeds the content of carotene may decrease between the time of collection and the time of analysis unless precaution is taken to preserve it, and losses may also occur during the preparation of the sample. Strain (4) found that fresh green leaves lose up to 93.8 per cent of their carotenoid pigments when they are ground with sand for 15 minutes and that the losses are retarded by heating the leaves at 90–

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<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1940, 562, 7(b), 1 or 2.

100°C.; by grinding the leaves in the presence of small quantities of zinc dust, zinc oxide, magnesium oxide, sodium hydroxide, ammonium hydroxide, copper sulfate, lead acetate, or mercuric chloride; and by grinding in the presence of large quantities of alcohol, ether, acetone, or chloroform.

The loss of carotene in grinding fresh samples may account for some of the peculiar results found in the literature, such as silage appearing to have more vitamin A potency than can be accounted for by its carotene content (5), and the supposed excretion of larger quantities of carotene than were ingested by cows (6). Methods of analysis for green feeds should be revised so as to prevent such losses.

#### PREVENTION OF LOSS OF CAROTENE DURING PREPARATION

Cooking the fresh sample without grinding and preserving with ethanol while grinding seemed to offer the most promising solutions to the problem. After some preliminary work, the following procedure was used in studying the effect upon the pure carotene content of the fresh material of grinding, of cooking and then grinding, and of grinding in ethanol.

From 500 grams of the fresh green material were weighed 3 portions of 100 grams each, selected to be as uniform as possible. One portion was ground in a food chopper, and any liquid that separated was added to the solid portion; 5 grams was taken for analysis. A second portion was cooked with about 200 ml. of water for 5 minutes, or longer if necessary for thorough cooking. Most of the liquid was pressed out through cheese cloth. The solids were weighed and ground in the food chopper, and a 1/20 aliquot was taken for analysis. The liquid was made up to 200 ml., and 10 ml. (1/20) was added to the aliquot of the solids, which gave a sample equivalent to 5 grams of the original material. To the third portion 100 ml. of 95 per cent ethyl alcohol was added and mixed in, and the material was cut into small pieces with scissors. The solid material was kept covered with alcohol. Sand (100 grams) was added, and the mixture was ground until a good preparation was obtained. Some samples absorbed all the alcohol. If such absorption did not occur and the liquid separated, it was pressed out through a cheese cloth. The solid portion was weighed, and a 1/20 aliquot was taken. The liquid was diluted to 200 ml., and 10 ml. was added to the solid aliquot.

Crude and pure carotene was determined on the three aliquots by the A.O.A.C. procedure as modified by the writers (2), and the results for pure carotene are given in Table 1. The carotene content is expressed in parts per million and is the average of two analyses on the same sample. For purposes of comparison, the carotene in the food treated with alcohol is put at 100 per cent, and that in the ground raw food and the cooked material is compared with it. Examination of the data shows that the carotene content of the samples ground in a fresh raw condition ranges from 63 per cent to 127 per cent of that of the samples treated with alcohol. Sweet potatoes, Bermuda grass, two out of three samples of carrots, and two out of five samples of spinach lost carotene on cooking. Cooking preserved the carotene to some extent in other materials worked on. The

alcohol treatment preserved the carotene better than did the cooking of Bermuda grass, sweet potatoes, spinach, and turnip greens. Cooking was better than or approximately equal to the alcohol treatment for the preservation of carotene in dried apricots, beet tops, bur clover, collards, green string beans, mustard greens, green bell peppers, parsley, and okra.

The high loss of carotene from sweet potatoes when cooked in the specified manner is in line with the results shown by Lease and Mitchell (3). These workers reported that saponification with alcoholic potassium hydroxide is not applicable in the determination of carotene in cooked sweet potatoes. They claim the hydrolytic products of starch formed on cooking react with alcoholic potassium hydroxide to form resins, which bind the carotene mechanically and render it unextractable with cold or boiling 95 per cent ethanol, ether, acetone, or petroleum benzin. In line with this an experiment was set up to develop a method for the determination of carotene in sweet potatoes. Sweet potatoes weighing 150 grams were peeled and divided into six approximately equal portions. These portions were analyzed as follows: No. 1 was boiled 30 minutes in 50 ml. of 95 per cent ethanol; No. 2 was boiled 30 minutes in 50 ml. of 12 per cent alcoholic potassium hydroxide; No. 3 was boiled for 15 minutes in 50 ml. of water and then 30 minutes more with 50 ml. of 95 per cent ethanol added; No. 4 was boiled for 15 minutes in 50 ml. of water and then 30 minutes more with 50 ml. of 12 per cent alcoholic potassium hydroxide added; and No. 5 was boiled for 30 minutes with 50 ml. of 12 per cent alcoholic potassium hydroxide and then 50 ml. of water added, and the entire contents were boiled for 15 minutes longer. Pure and crude carotene was determined on the five samples so prepared, care being taken to extract all coloring matter from the sweet potato residue after each treatment. Water was run on the sixth portion. Results are given in Table 2. This work shows that cooking either destroyed or rendered some of the carotene unextractable and also that saponification with alcoholic potassium hydroxide gave lower results than did the 95 per cent ethanol method of extraction. These higher results were thought to be due to the presence of xanthophyl esters, which can not be removed except by saponification. To determine whether sweet potatoes contain xanthophyls or their esters, three different samples of yellow sweet potatoes were boiled with 95 per cent ethanol and extracted with petroleum benzin as specified above. The petroleum benzin extracts were shaken in separatory funnels for 10-15 minutes with a 12 per cent solution of potassium hydroxide in methanol, water was added, and the methanol layers were drawn off. The petroleum benzin extracts were next shaken with 90 per cent methanol. No color was removed from the petroleum benzin by either of the above treatments, which shows that the sweet potatoes tested contained no detectable amounts of xanthophyl or xanthophyl esters.

TABLE 1.—*Pure carotene content of fresh foods as affected by grinding raw, cooking, or grinding in alcohol*

LABORATORY NUMBER	KIND OF MATERIAL	ON ORIGINAL FRESH BASIS			RELATIVE QUANTITY (ALCOHOL TREATED AS 100)	
		GROUND RAW	COOKED	TREATED WITH ALCOHOL	GROUND RAW	COOKED
55206	Apricots, dried	p.p.m. 65.6	p.p.m. 75.2	p.p.m. 74.8	88	101
58336	Beans, green string	3.1	3.9	3.4	91	115
55208	Beet tops	32.2	44.8	38.2	84	117
57765		34.8	38.0	39.5	88	96
55237		32.2	31.6	31.8	101	99
56054	Bermuda grass	148.0	111.6	158.4	93	70
56423		140.0	134.0	152.5	92	88
56045	Bur clover	58.0	64.0	63.0	92	102
56443		60.6	76.4	71.0	85	108
55118	Carrots	83.7	88.0	84.8	99	104
57756		45.4	39.4	35.7	127	110
55127	Collards	38.4	54.2	41.0	94	132
55067	Mustard greens	31.4	42.0	35.6	88	118
56567		24.2	45.4	38.6	63	118
55116		28.0	29.6	31.4	89	94
58032		18.6	22.2	21.8	85	102
58333	Okra	2.8	2.7	2.8	100	96
55209	Parsley	49.2	50.6	53.1	93	95
57947		31.5	31.2	30.4	104	103
55115	Peppers, green	2.8	3.5	2.7	104	130
55207		2.3	3.0	2.8	82	107
55119	Potatoes, sweet	57.2	21.4	58.4	98	37
55124		45.0	39.0	54.2	83	72
55236	Spinach	40.8	35.8	37.6	109	95
55083		36.0	44.0	41.8	86	105
55889		43.4	38.4	49.2	88	78
57766		47.5	50.5	57.0	83	89
58172		47.3	55.0	57.0	83	96
55054	Turnip greens	34.8	47.2	50.0	70	94
56902		39.8	41.8	52.8	75	79
57026		53.0	61.0	79.0	67	77

TABLE 2.—*Effect of cooking and of alcoholic potash on carotene in sweet potatoes*

LABO- RATORY NUMBER	ORIGINAL FRESH BASIS, CAROTENE P.P.M.					RELATIVE QUANTITY ALCOHOL TREATED AS 100			
	RAW BY ETHANOL	RAW BY 12% ALCOHOLIC KOH	COOKED BY ETHANOL	COOKED BY 12% ALCOHOLIC KOH	RAW BY 12% ALCOHOLIC POTASH BOILED IN 3 VOLUMES WATER	RAW ALCOHOLIC KOH	COOKED ETHANOL	COOKED ALCOHOLIC KOH	RAW ALCOHOLIC KOH BOILED
57557	47.7	29.9	39.0	42.5	39.2	63	82	89	82
57733	32.1	38.0	47.2	16.1	35.9	118	147	50	112
57772	39.8	33.2	35.1	25.8	13.7	83	88	65	34
57929	46.8	28.9	34.6	26.7	17.5	62	74	57	37

## PRESERVATION OF CAROTENE BEFORE PREPARATION OF SAMPLE

Taking green samples to the laboratory immediately and cooking them at once is not always practical. Some additional method is needed to preserve the carotene until the analysis can be made.

Fresh samples of Bermuda grass and bur clover were stored for 7 days in methanol, 50 per cent methanol, 3 per cent formaldehyde, 1 per cent boric acid, 0.5 per cent salicylic acid, and 0.1 per cent mercuric bichloride. There were losses of carotene with all except methanol. With bur clover the highest loss was with mercuric bichloride, but high losses also occurred with formaldehyde and boric acid. The losses with 50 per cent methanol and 0.5 per cent salicylic acid were small. With fresh Bermuda grass the results were similar. There were small losses, perhaps in the limit of error, with 50 per cent methanol and 0.5 per cent salicylic acid.

The method proposed for the determination of carotene in fresh green materials is as follows:

## PROPOSED METHOD

For samples to be analyzed soon after gathering (1–2 hours), place 100 grams of material in a large evaporating dish, soak in 100 ml. of 95 per cent ethyl alcohol for 5 minutes, and cut up with scissors. Add 100 grams of clean white sand, free of organic matter, and grind until a uniform mixture is obtained. For samples necessitating shipment or delay in analysis, place approximately 100 grams of material in a tared fruit jar with a weighed quantity of alcohol. Seal the jar by using a jar rubber under the lid. Upon arrival at laboratory, weigh the jar and contents. Subtract weight of jar and alcohol from total weight to give weight of sample. Pour contents of jar into an evaporating dish, cut up with scissors, and grind with sand as directed above. Decant liquid from solid part through a cheese cloth and make up to volume. Weigh solids. Take aliquots of both solid and liquid equivalent to 5 grams of the fresh untreated material and mix together for carotene analysis. Saponify the sample by boiling for 30 minutes in 50 ml. of 12 per cent alcoholic KOH. Cool, add 50 ml. of petroleum benzin, and decant liquid into a separatory funnel. Transfer residue to a mortar, grind with a pestle, first with a 15 ml. portion of petroleum benzin and then with a mixture of 5 ml. of 95% ethyl alcohol and 15 ml. of petroleum benzin until no further color is extracted. Then proceed as directed in the A.O.A.C. method for

crude carotene (7). For sweet potatoes follow the same procedure, except to boil for 30 minutes with 50 ml. of 95% ethanol, instead of 50 ml. of 12 per cent alcoholic KOH.

For pure carotene, shake 50 ml. of crude carotene solution with 2.5 grams of activated  $MgCO_3$ , X reagent (1). (This method is applicable to green leafy vegetables and feeds.)

#### SUMMARY

Considerable loss of carotene occurred when fresh foods (with the exception of parsley, okra, and carrots) were ground raw for analysis. This loss was inhibited in most of the foods by cooking them before grinding or by grinding them while they were covered with alcohol. The fresh samples after grinding contained 63–127 per cent of the carotene contained in the alcohol-treated samples. Samples treated by cooking contained 37–132 per cent of the carotene contained in the alcohol treated samples. The carotene content of sweet potatoes, Bermuda grass, two out of three samples of carrots, and two out of five samples of spinach was less in cooked material than in the material ground while uncooked.

Fresh Bermuda grass or bur clover stored for 7 days in methanol lost only slight amounts of carotene.

Methods for preservation and preparation of green foods are given.

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#### COMPOSITION OF THE ASH OF DRY SKIM MILK AND ITS RELATION TO NEUTRALIZATION

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In his report as Associate Referee on Neutralizers in Dairy Products,<sup>1</sup> presented last year to the Association of Official Agricultural Chemists attention was called to the fact that sodium bicarbonate and sodium

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<sup>1</sup> See p. 556.

carbonate, when added to a dry skim milk, were only partially returned as alkalinity of the water-soluble ash.

When a milk is neutralized, basic sodium, calcium, and magnesium compounds, either singly or in combination with each other, are usually employed. Since most milk becomes sour owing to the formation of lactic acid through bacterial decomposition, sodium, calcium, or magnesium lactates would be formed on neutralization with these bases. If a milk so neutralized with sodium were ashed, it might be expected that the added sodium would be returned in the water-soluble ash as sodium carbonate, with possible traces of the oxide. Likewise, if calcium were used as the medium for neutralization no increase in the water-soluble alkalinity through solubility of calcium carbonate would be expected (Wichmann<sup>2</sup> has shown that there is little or no formation of calcium oxide at a temperature of 550°C.).

In order to duplicate, as closely as possible, conditions existing in a neutralized milk, the writer added sodium lactate and calcium lactate in known amounts to a reconstituted, unneutralized, dry skim milk. These liquids were then ashed at 550°C., and the alkalinity of the total ash and of the water-soluble ash was determined according to the methods given in the report referred to.<sup>1</sup> The results are presented in Tables 1 and 2. "Alkalinity" in the first column of each of the tables represents the alkalinity of the quantity of sodium or calcium lactate added in each case. These calculated "alkalinities" are based on a determined alkalinity of the prepared solution added in known amounts to 2 grams of dry skim milk.

The surprising results previously obtained<sup>1</sup> on the determination of the alkalinity of the water-soluble ash when sodium carbonate and bicarbonate were added to a reconstituted dry skim milk indicated that all the sodium lactate added would not be returned as alkalinity of water-soluble ash (Table 1). It is of interest to note that after an addition of sodium lactate of an alkalinity equivalent to 12 ml. of 0.1 *N* a loss of approximately 4.5 ml. was found, and any further increase in the quantity of sodium lactate added produced no significant increase in the loss. Excellent recoveries of added sodium lactate, calculated from the alkalinity of the total ash, were obtained.

Results obtained on the alkalinity of the water-soluble ash of reconstituted dry skim milk to which calcium lactate had been added were equally as contrary to theory as those obtained on the addition of sodium salts. Since insoluble calcium carbonate is formed on ashing calcium lactate, its addition would not be expected, *a priori*, to increase the water-soluble alkalinity of the ash of a normal dry skim milk. Reference to

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<sup>2</sup> This Journal, 24, 441 (1941).



TABLE 1.—*Recoveries of added sodium lactate calculated from alkalinities (ml. 0.1 N)*

"ALKALINITY" OF SODIUM LACTATE ADDED	ALKALINITY OF WATER- SOLUBLE ASH	ALKALINITY OF WATER- SOLUBLE ASH CORRECTED FOR THAT OF ORIG. MILK	ALKALINITY OF WATER- SOLUBLE ASH	LOSS IN THEORETICAL WATER- SOLUBLE ALKALINITY	ALKALINITY OF TOTAL ASH	ALKALINITY OF TOTAL ASH CORRECTED FOR THAT OF ORIG. MILK (RECOVERY)	RECOVERY OF ADDED SODIUM LACTATE
			<i>per cent of theory</i>				<i>per cent of theory</i>
0.00	0.50	—	—	—	2.00	—	—
1.08	0.76 0.72	0.26 0.22	22.2	0.84	3.20 3.24	1.20 1.24	113.0
2.15	0.77 0.74	0.27 0.24	12.1	1.89	4.15 4.35	2.15 2.35	104.6
4.30	1.60 1.49	1.10 0.99	24.6	3.25	6.15 6.45	4.15 4.45	100.0
6.45	2.84 3.06	2.34 2.56	38.0	4.00	8.49 8.35	6.49 6.35	100.0
8.60	3.97 4.15	3.47 3.65	41.6	4.04	10.55 10.55	8.55 8.55	99.5
12.62	8.51 8.80	8.01 8.30	64.6	4.44	14.55 14.45	12.55 12.45	99.1
15.14	11.00 10.65	10.50 10.15	68.2	4.81	16.65 16.85	14.65 14.85	97.4
17.30	13.23 13.53	12.73 13.03	74.4	4.42	18.95 19.15	16.95 17.15	98.5
18.93	14.72 14.98	14.22 14.48	75.8	4.58	20.95 20.75	18.95 18.75	99.5
22.71	18.26	17.76	78.2	4.94	24.55 24.45	22.55 22.45	99.1
25.34	21.47 21.34	20.97 20.84	82.5	4.43	27.33 27.28	25.33 25.28	99.9

Table 2, however, will show that such an increase actually took place in the alkalinity of the water-soluble ash of a milk to which varying quantities of calcium lactate had been added prior to evaporation and ashing.

TABLE 2.—*Recoveries of added calcium lactate calculated from alkalinities (ml. 0.1 N)*

"ALKALINITY" OF CALCIUM LACTATE ADDED	ALKALINITY OF WATER- SOLUBLE ASH	ALKALINITY OF WATER-SOLUBLE ASH CORRECTED FOR THAT OF ORIG. MILK INCREASE OVER THEORETICAL	ALKALINITY OF TOTAL ASH	ALKALINITY OF TOTAL ASH CORRECTED FOR THAT OF ORIG. MILK (RECOVERY)	RECOVERY OF ADDED CALCIUM LACTATE
0.00	0.50	—	2.00	—	<i>per cent of theory</i> —
1.93	0.88 0.77	0.38 0.27	4.05 4.15	2.05 2.15	108.8
3.85	1.42 1.21	0.92 0.71	5.85 5.95	3.85 3.95	101.3
5.78	2.34 2.15	1.84 1.65	7.84 7.95	5.84 5.95	102.1
7.70	3.41 3.25	2.91 2.75	9.55 9.60	7.55 7.60	98.5
9.80	4.28 3.96	3.78 3.46	11.55 11.35	9.55 9.35	96.4
11.55	4.31 4.60	3.81 4.10	13.35 12.95	11.35 10.95	95.7
13.50	4.79 4.68	4.29 4.18	15.42 15.70	13.42 13.70	100.4
15.40	4.35 4.62	3.85 4.12	17.05 16.95	15.05 14.95	97.4
17.33	4.40	3.90	—	—	—
19.04	4.40	3.90	—	—	—
27.00	4.40	3.90	—	—	—

This increase became fairly constant after about 10 ml. of 0.1 *N* calcium lactate had been added.

Figure 1 is a graphical presentation of the data in Tables 1 and 2.

A study was also made of the effect produced by the addition of both sodium and calcium lactate on the distribution of sodium and potassium between the water-soluble and water-insoluble ash of reconstituted dry

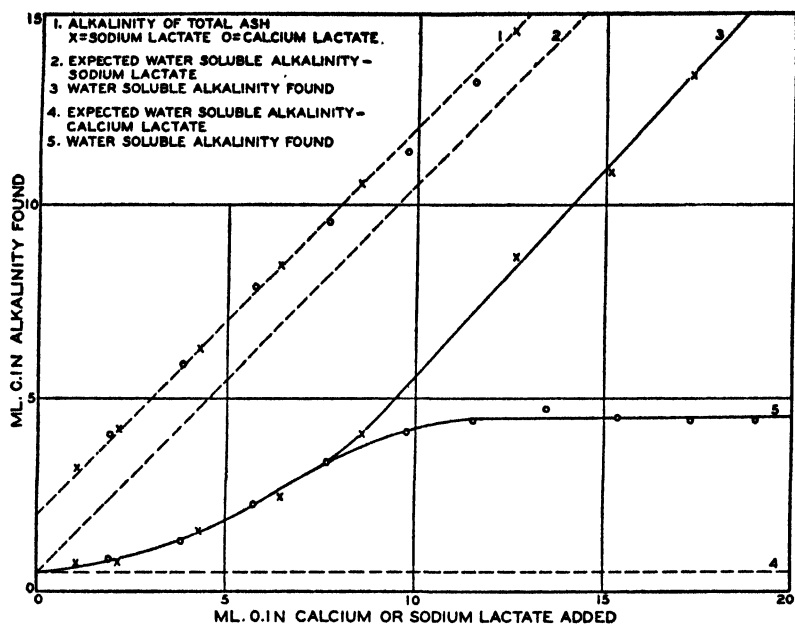


FIG. 1.

skim milk. Data showing the effect on sodium distribution of the addition of sodium lactate are presented in Table 3. Sodium was determined by the uranyl acetate method.<sup>3</sup>

TABLE 3.—Effect of addition of sodium lactate on distribution of sodium in milk ash (mg.)

SODIUM LACTATE ADDED IN TERMS OF Na <sub>2</sub> O	Na <sub>2</sub> O FOUND IN TOTAL ASH	Na <sub>2</sub> O FOUND IN TOTAL ASH CORRECTED FOR THAT IN ORIG. MILK	Na <sub>2</sub> O FOUND IN WATER-SOLUBLE ASH		Na <sub>2</sub> O FOUND IN WATER-INSOLUBLE ASH		NET RECOVERY OF Na <sub>2</sub> O IN WATER-SOLUBLE AND WATER- INSOLUBLE ASH
			TOTAL	CORRECTED FOR THAT IN ORIG. MILK	TOTAL	CORRECTED FOR THAT IN ORIG. MILK	
0.0	14.2	—	1.7	—	11.8	—	—
11.9	26.3	12.1	7.2	5.5	18.8	7.0	12.5
18.3	32.8	18.6	14.1	12.4	17.9	6.1	18.5
30.7	45.4	31.2	24.5	22.8	20.7	8.9	31.7
36.6	52.4	38.2	29.9	28.2	21.0	9.8	38.0
44.6	60.2	46.0	36.7	35.0	22.9	11.1	46.1
62.6	76.7	62.5	53.9	52.2	21.9	10.1	62.3
80.0	92.7	78.5	70.1	68.4	21.5	9.7	78.1

The sodium found in both the water-soluble ash and the water-insoluble ash checks that found in the total ash. An appreciable quantity of added

<sup>3</sup> *Methods of Analysis*, A.O.A.C., 1940, 130.

sodium is contributed to the water-insoluble ash, which explains the fact, previously noted, that the recovery of added sodium lactate is low when calculated from the water-soluble ash alkalinity. Again, as with soluble alkalinity, there is stabilization after about 10 ml. of 0.1 *N* sodium lactate (equivalent to about 31 mg. of sodium oxide) has been added.

Data showing the effect on the distribution of potassium of the addition of sodium lactate are presented in Table 4. Potassium was determined by the sodium cobaltinitrite method.

TABLE 4.—*Effect of addition of sodium lactate on distribution of potassium in milk ash (mg.)*

SODIUM LACTATE IN TERMS OF Na <sub>2</sub> O	K <sub>2</sub> O			
	FOUND IN WATER-SOLUBLE ASH	FOUND IN WATER-INSOLUBLE ASH	TOTAL FOUND IN WATER-SOLUBLE AND WATER-INSOLUBLE ASH	FOUND IN TOTAL ASH
—	32.1	6.3	38.4	39.1
18.3	33.8	5.7	39.5	38.6
36.6	34.6	4.3	38.9	39.2
44.6	36.5	3.6	40.1	39.7
62.6	36.3	3.4	39.7	39.8
80.0	38.4	1.2	39.6	39.3

Most of the potassium normally present in milk is found in the water-soluble ash, whereas, as stated previously, most of the sodium is found in the water-insoluble ash. The potassium in the water-insoluble ash was found to decrease steadily with the addition of increasing quantities of sodium lactate. This is reflected by an increase in the potassium found in the water-soluble ash. The potassium found in both the water-soluble and water-insoluble ash checks that found in the total ash very closely.

Data showing the effect on the distribution of sodium and potassium through the addition of calcium lactate are presented in Table 5.

TABLE 5.—*Effect of addition of calcium lactate on distribution of sodium and potassium in milk ash (mg.)*

CALCIUM LACTATE ADDED IN TERMS OF CaO	Na <sub>2</sub> O		K <sub>2</sub> O	
	FOUND IN WATER-SOLUBLE ASH	FOUND IN WATER-INSOLUBLE ASH	FOUND IN WATER-SOLUBLE ASH	FOUND IN WATER-INSOLUBLE ASH
—	1.7	11.8	32.1	6.3
16.2	6.0	6.6	35.5	3.8
21.6	7.7	5.4	35.9	1.6
32.3	10.1	3.2	38.2	None
53.9	10.1	3.6	39.0	None

It will be seen that the calcium replaced most of the sodium and all of the potassium present in the insoluble ash. The sodium and potassium replaced appeared in the water-soluble ash, and this accounts for the increase in water-soluble alkalinity previously noted (Table 2). All the potassium and most of the sodium became soluble after the addition of about 11 ml. of 0.1 *N* calcium lactate. This again is in line with the observation that the water-soluble alkalinity chargeable to added calcium lactate became fairly constant after the addition of about 10 ml. of 0.1 *N* calcium lactate.

Data are presented in Table 6 showing that all the sodium and potassium in the water-soluble ash of "neutralized" milk do not appear in the alkalinity titration.

TABLE 6.—*Distribution of sodium and potassium in the water-soluble ash of milk (ml. of 0.1 N)*

SODIUM LACTATE ADDED	A Na <sub>2</sub> O FOUND	B K <sub>2</sub> O FOUND	TOTAL A+B	C ALKALINITY	D CHLORIDES FOUND	E SULFATES FOUND	F PHOSPHATES FOUND	TOTAL C+D+E+F
—	0.5	6.8	7.3	0.5	6.3	0.6	0.2	7.6
11.8	9.6	7.4	17.0	8.0	6.6	0.6	1.9	17.1
20.2	18.7	7.7	26.4	16.1	6.8	0.7	2.5	26.1
25.8	22.6	8.2	30.8	20.5	6.8	0.9	3.1	31.3

Varying quantities of sodium lactate were added to the milk, and sodium and potassium were determined in the water-soluble ash. These determinations are expressed in the table as ml. of 0.1 *N* oxide. The alkalinity of the water-soluble ash was determined as were also the quantities of chlorides, sulfates, and phosphates present. It will be seen (Table 6) that only a portion of the sodium and potassium in the water-soluble ash contributes to the alkalinity. However, when the quantities of chlorides, sulfates, and phosphates found are added to the alkalinity it will be seen that this total closely checks the sum of the sodium and potassium found. Of particular interest are the small amounts of sodium and potassium contributing to the water-soluble alkalinity of the milk itself as compared to the sodium and potassium that are combined as the chloride. Also, it is of interest to note that as the quantity of added sodium lactate was increased, the phosphates became somewhat more soluble.

The data in Tables 3, 4, 5, and 6 show the effect of added sodium and calcium lactate on the distribution of sodium and potassium in the water-soluble and water-insoluble ash of milk. No attempt will be made to explain the phenomena presented, but unquestionably a base exchange took place, and the phosphates figure prominently in the reactions. Un-

doubtedly sodium and potassium are combined with the phosphates in some form of complex salt.

#### SUMMARY

Data showing that the alkalinity of the water-soluble ash does not give a true measure of the sodium and potassium normally present in the ash are given. Part of the sodium added to milk as sodium lactate appears in the water-insoluble ash. The addition of calcium lactate caused an unsuspected increase in the alkalinity of the water-soluble ash. The alkalinity of the water-soluble ash, therefore, measures neither the total sodium and potassium present in milk ash itself nor the basic sodium or calcium compounds present in milk as a result of neutralization. The increase in total alkalinity above normal is, however, a good measure of the amount of such neutralizing compounds used.

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#### ANALYSIS OF MIXTURES OF FD&C YELLOW NO. 3 AND D&C GREEN NO. 6

By J. A. KIME\* (Cosmetic Division, U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Often the determination of the total pure dye content in mixtures of coal-tar colors offered for certification is rather easily made. The sample, dissolved in an appropriate solvent, is titrated with titanium trichloride in the presence of a suitable buffer catalyst. From the amount of reducing agent consumed, the percentage of pure dye is calculated by means of a factor based on composition of the mixture as revealed by the manufacturer in his request for certification.

Mixtures of FD&C Yellow No. 3 (formerly Yellow AB) and D&C Green No. 6 (formerly Quinizarin Green), either dry or dissolved in benzyl alcohol, are certifiable for use in drugs and cosmetics. The properties of these colors, however, are so dissimilar that no one set of conditions is now available for their quantitative reduction in the presence of each other. The solubility of D&C Green No. 6 is too low in aqueous, aromatic, solvent mixtures that contain sufficient water for the solution of a buffer catalyst to permit determination of pure dye by reduction with titanium trichloride. This factor might be overcome if the reduction products did not precipitate and mask the end point. In the analysis of the straight color itself the solubility is increased by sulfonation with concentrated sulfuric acid, and the quantitative reduction proceeds smoothly in approximately 50 per cent alcohol with a sodium citrate buffer catalyst. The reduction products then remain in solution. If, however, mixtures of dry D&C Green

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No. 6 and FD&C Yellow No. 3 are treated with sulfuric acid a large percentage of the azo component is decomposed. It is possible that special sulfonating agents could be used to increase the solubility of the D&C

TABLE 1.—*Properties of the colors used*

	FD&C YELLOW NO. 3	D&C GREEN NO. 6
Former designation	Yellow AB	Quinizarin Green
Colour index no.	22	1078
Approximate solubilities at 25°C.		
In benzyl alcohol	More than 10% w/v	0.25% w/v
In 95% ethyl alcohol	3.7% w/v	0.01% w/v
In 60% ethyl alcohol	0.27% w/v	Less than 0.002% w/v
In water	Insoluble	Insoluble
Pure dye, as calculated from nitrogen content	—	98.5%
Pure dye, as determined by titration with $\text{TiCl}_3$	99.6%	98.9%
No. of hydrogen equivalents per mol required for reduction	4	2
Gram of pure dye per ml. 0.1 N $\text{TiCl}_3$	.006180	.02091
Volatile matter		
At 80°C.	0.09%	—
At 135°C.	—	0.27%

TABLE 2.—*Recoveries on prepared mixtures*

MIX-TURE NO.	COLOR	DILUENT IN MIXTURE	SAMPLE	PURE DYE		RECOVERY
				PRESENT	FOUND	
1	D&C Green No. 6 FD&C Yellow No. 3	None	gram 1.000	gram .740	gram .748	per cent 101.1
				.249	.249	100.0
2	D&C Green No. 6 FD&C Yellow No. 3	Benzyl Alcohol	50.00	.124	.1194	96.2
				.125	.127	101.6
3	D&C Green No. 6 FD&C Yellow No. 3	Benzyl Alcohol	50.00	.100	.0942	94.2
				.150	.1523	101.5
4	D&C Green No. 6 FD&C Yellow No. 3	None	0.370	.365	.368	100.8
				—	—	—

Green No. 6 without decomposing the FD&C Yellow No. 3 but then it would be necessary to find a suitable buffer catalyst. Among the catalysts that have been tried only sodium citrate provides proper conditions for the reduction of the green color, and it was definitely unsuitable for quantitative reduction of the yellow color.

In liquid mixtures it might be necessary to separate the colors from the solvent before sulfonation. The conditions for such separation and for an individual estimation of the two components have been determined. Colors of certified grade were used for these experiments. The buffers were of C.P. quality and 0.1 *N* titanium trichloride was prepared and standardized in the usual manner.<sup>1</sup> The dye solutions were agitated mechanically under an atmosphere of carbon dioxide during reduction. The properties and methods of analysis of FD&C Yellow No. 3 are known.<sup>1</sup> The method for determining the pure dye in the sample of D&C Green No. 6 by titration with titanium trichloride has not been described. It follows:

#### PURE DYE IN D&C GREEN NO. 6

Transfer or weigh direct into a 23×150 mm. test tube an accurately weighed sample aliquot of ca. 0.35 gram. Wash down the wall of the test tube with 4 ml. of concentrated  $H_2SO_4$ , cover with a watch-glass, and heat in the steam bath for 30 minutes. (A convenient support may be made by cutting a one-quarter inch layer from No. 10 rubber stopper and boring a 20 mm. hole.) Wash down the wall of the test tube with 1–2 ml. of concentrated  $H_2SO_4$  and continue heating for 1 hour. Remove the test tube, cool, and pour contents into 100 ml. of 50% alcohol in a 1 liter extraction flask. Partly neutralize with 5 grams of  $Na_2CO_3$  and add 150 ml. of 50% alcohol and 15 grams of sodium citrate. Heat to boiling and titrate with 0.1 *N*  $TiCl_3$  under an inert atmosphere. (The end point is a color change from a turbid greenish yellow to a clear yellow.) Calculate the percentage of pure dye from the factor given in Table 1. (That 2 hydrogen equivalents are required to reduce 1 mole of dye is substantiated by the agreement of pure dye values in Table 1, calculated from nitrogen determination and from titrimetric reduction.)

#### Analytical Method for Mixtures

Dilute 0.5–1.0 gram of dry mixture or 25–50 grams of liquid mixture to 150 ml. with 95% ethyl alcohol. Heat on the steam bath for 30 minutes, add 80 ml. of water with stirring, and let stand overnight. Filter off the D&C Green No. 6 on a tared Gooch crucible, rinse the beaker, and wash the residual FD&C Yellow No. 3 from the crucible with 40% alcohol. Dry the precipitate at 135°C. for 3 hours, cool in a desiccator, weigh, and calculate the percentage of D&C Green No. 6. Transfer the filtrate to a 500 ml. volumetric flask and dilute to the mark with alcohol. Pipet a 100 ml. aliquot (200 ml. with small amounts of FD&C Yellow No. 3) into a 500 ml. wide-mouthed Erlenmeyer flask; add 100 ml. of water, 15 grams of sodium bitartrate, 1 ml. of a 1% light green SF yellowish indicator solution; heat to almost boiling; and titrate with  $TiCl_3$  under an inert atmosphere. Deduct the blank due to indicator and calculate from net ml. of  $TiCl_3$  consumed, the percentage of FD&C Yellow No. 3, using the factor given in Table 1.

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1940, 259–260.



## DISCUSSION

The recoveries recorded in Table 2 indicate that separation is less precise in Mixtures 2 and 3. The solubility effect on the green color is perceptible at approximately 25 per cent concentration of the benzyl alcohol. The filtrates in Mixtures 2 and 3 were greenish, whereas in Mixture 1 the filtrate was almost a clear yellow.

In general, however, the precision is satisfactory for dye analysis work and the method applicable to the determination of the pure dye content of mixtures of D&C Green No. 6 and FD&C Yellow No. 3.

## SUMMARY

Methods are presented for the determination of pure dye in the straight color D&C Green No. 6 and in mixtures of this color with FD&C Yellow No. 3. Typical results are given.

## DETECTION AND DETERMINATION OF MONO- AND DI-ETHANOLAMINES

By IRWIN S. SHUPE (Cosmetic Division,\* U. S. Food and Drug Administration, Federal Security Agency, Baltimore, Md.)

Mono-, di- and tri-ethanolamines are used as emulsifying agents in the preparation of creams, lotions, and other cosmetics. They are primary, secondary and tertiary amines, respectively, and have basic salt-forming properties.

<i>Name</i>	<i>Formula</i>	<i>Mol. Wt.</i>	<i>Boiling Point<sup>1</sup></i>
Monoethanolamine (2-hydroxyethylamine)	$\text{NH}_2\text{-CH}_2\text{-CH}_2\text{OH}$	61	172° C. †
Diethanolamine (2,2' dihydroxydiethylamine)	$\text{NH}(\text{CH}_2\text{-CH}_2\text{OH})_2$	105	268° C.
Triethanolamine (Trihydroxytriethylamine)	$\text{N}(\text{CH}_2\text{-CH}_2\text{OH})_3$	149	279° C. (150 mm.)

In order to overcome the discoloration in creams caused by triethanolamine, the mono and di compounds have been introduced. Their lower equivalent weights also permit the use of smaller quantities. Monoethanolamine sulfite has been proposed for use in hair-waving preparations.<sup>2</sup>

Methods have been devised for triethanolamine,<sup>3</sup> but apparently none has been published for the mono and di compounds. A study has been made of mono- and di-ethanolamines, their properties and analysis.

\* D. Dahle, in charge.

<sup>1</sup> The Merck Index, 5th Ed. (1940), Merck and Co., Inc., Rahway, N. J.

<sup>2</sup> *Am. Perfumer*, 41, 40 (1940).

<sup>3</sup> *Analyst* 60, 77 (1935); 62, 261 (1937).

## METHODS

*Color tests.*—To a solution containing about 0.2% monoethanolamine, add an equal volume of a solution composed of 1 gram of sodium nitroprusside and 20 ml. of acetone in water to make 100 ml. Add sufficient 2%  $\text{NaHCO}_3$  solution to assure an alkaline reaction. A purple color gradually develops in the presence of the primary amine.

To test for diethanolamine, make the test as above, but use 10 ml. of acetaldehyde instead of acetone in preparing the reagent. A blue color is formed with the secondary amine.

*p-Brombenzene sulfonyl derivatives.*—Place 10 ml. of a slightly acid solution of sample containing not over 100 mg. of the ethanolamines in a 250 ml. beaker. Dissolve 1 gram of reagent *p*-brombenzene sulfonyl chloride in 15 ml. of acetone and add to the aqueous solution of the sample. Then add approximately 1 gram of powdered  $\text{NaHCO}_3$ . Cover the beaker with a watch-glass and heat on a steam bath until the acetone has volatilized (15–20 minutes). Add 10 ml. of 10% w/v  $\text{NaOH}$  and 10 ml. of ethyl alcohol. Heat again on the steam bath and evaporate to about 20 ml. to remove the alcohol (ca. 30 minutes).

*Diethanolamine.*—Let cool and transfer to separatory funnel No. 1. Wash the beaker with ca. 5 ml. of water. Extract the contents of funnel No. 1 with four 20 ml. portions of  $\text{CHCl}_3$ . Combine these four extracts in separatory funnel No. 2 and wash them with 10 ml. of 10% w/v  $\text{NaOH}$ . Draw off the  $\text{CHCl}_3$  layer into separatory funnel No. 3 (saving the  $\text{NaOH}$ ), wash the  $\text{CHCl}_3$  with 10 ml. of water, and filter through a pledget of cotton into a tared dish. Save the wash water. Repeat the procedure with two additional 20 ml. portions of  $\text{CHCl}_3$ , using the  $\text{NaOH}$  and the water in separatory funnels Nos. 2 and 3 for the washing. Add these  $\text{CHCl}_3$  extracts to the contents of the tared dish, evaporate on a steam bath, dry in a desiccator, and weigh the *p*-brombenzene sulfonyl diethanolamine.

*p*-Brombenzene sulfonyl derivative  $\times 0.324$  = diethanolamine.

Reserve the contents of the three separatory funnels for the monoethanolamine determination.

*Monoethanolamine.*—Combine the contents of separatory funnels Nos. 1 and 2. Acidify with 10 ml. of concentrated  $\text{HCl}$  and extract with six 25 ml. portions of  $\text{CHCl}_3$ . Acidify the wash water in separatory funnel No. 3 with a drop of 10%  $\text{HCl}$  and use it for washing the  $\text{CHCl}_3$  extracts. Filter through cotton, evaporate, and dry as before. Weigh the *p*-brombenzene sulfonyl monoethanolamine.

*p*-Brombenzene sulfonyl derivative  $\times 0.218$  = monoethanolamine.

Make a blank determination on 1 gram of the reagent *p*-brombenzene sulfonyl chloride and apply appropriate corrections.

Both derivatives may be recrystallized from  $\text{CHCl}_3$  and petroleum benzin.

Melting point of diethanolamine derivative— $105^\circ \text{C}$ .

Melting point of monoethanolamine derivative— $94^\circ \text{C}$ .

## DISCUSSION

Samples of mono- and di-ethanolamines purchased on the open market showed purities of 98.1 and 98.9 per cent, respectively, by titration with acid. They were considered sufficiently pure to use as standards in developing the methods proposed here. The oxalate of monoethanolamine was prepared from the base and oxalic acid, and crystallized from alcohol. It melted at  $203^\circ \text{C}$ . with effervescent decomposition. No pure crystalline salt suitable for a standard was obtained with diethanolamine. The

hydrochloride, sulfate, and phosphate salts of the bases are hygroscopic and separate as liquids.

The color tests are modifications of Rimini's<sup>4</sup> test for primary aliphatic amines and Simon's test<sup>5</sup> for secondary amines.

The crystalline precipitates obtained with Kraut's reagent and diethanolamine, and with phosphotungstic acid and both the mono- and di-amines are useful as microchemical tests.

Volatilization with steam and extraction with immiscible solvents were unsuitable procedures for isolation of the ethanolamine bases. Monoethanolamine in the presence of 25 per cent sodium hydroxide was very slowly volatile with steam and partly extractable with ether. Diethanolamine under like conditions was not volatilized or extracted.

Several derivatives of the ethanolamines were either water soluble or liquids and thus were considered unsatisfactory for identity tests. Acetyl and chloracetyl derivatives were water soluble; dithiocarbamates, formed with carbon disulfide, were liquids. The benzene sulfonyl derivative of diethanolamine was a solid melting at 130° C., but the monoethanolamine derivative was a liquid.

TABLE 1.—*Recovery of p-brombenzene sulfonyl derivatives of mono- and di-ethanolamine*

EXP. NO.	ETHANOLAMINE	WEIGHT	WT. OF DERIVATIVE	RECOVERY OF BASE
		mg.	mg.	per cent
1	Di-	25.7	77.2	97.0
2	Di-	51.4	157.5	99.4
3	Di-	102.8	315.2	99.2
4	Mono-	26.3	123.0	102.0
5	Mono-	52.6	242.6	100.5
6	Mono-	105.2	486.8	100.8
7	Mono-	52.6	238.5	99.0
	Di-	51.4	160.0	101.0

TABLE 2.—*Analytical results from application of the proposed methods to other products*

MATERIAL	DIETHANOLAMINE	MONOETHANOLAMINE
	per cent	per cent
Commercial triethanolamine	5.1	3.0
Commercial monoethanolamine borate		51.8
Monoethanolamine oxalate (M.P. 203° C.)		58.0
		(Theory—57.6)

*p*-Brombenzene sulfonyl chloride, first proposed by Marvel and Smith,<sup>6</sup> as a reagent for amines, was found to yield crystalline derivatives of

<sup>4</sup> Mulliken, Identification of Pure Organic Compounds, Vol. 2, p. 35 (1916). John Wiley and Sons, Inc., N. Y.

<sup>5</sup> *Ibid.*, p. 43, or Feigl, Spot Tests, 2nd English ed., p. 307 (1939). Nordemann Pub. Co., Inc., N. Y.

<sup>6</sup> *J. Am. Chem. Soc.*, 45, 2696 (1923).

definite melting points with both the mono- and di-ethanolamines. The mono derivative was readily soluble in alkali, while the diethanolamine derivative was insoluble. This permits the separation of the mono- and di-ethanolamines when both are present in aqueous solutions. The triethanolamine, a tertiary amine, does not react with this reagent.

The methods are designed to minimize the formation of the disulfonyl monoethanolamine (M.P. 185° C.), which is insoluble in alkali and might be mistaken for a secondary amine derivative.

For the recovery of the ethanolamines from emulsions, such as cosmetic creams, an aqueous extract may be used. Substances extractable with chloroform, which would interfere in the method, may be removed by preliminary extractions from either acid or alkaline solutions before the preparation of the derivative. The ethanolamine bases remain in the aqueous portion. Ammonia should not be added since it forms a sulfonamide corresponding to a primary amine derivative.

Table 1 illustrates some recovery data on solutions of known concentration. In Table 2 are listed the results obtained from the application of the proposed methods to some commercial products and to a prepared specimen of monoethanolamine oxalate.

#### SUMMARY

Methods applicable to cosmetics are suggested for the detection and determination of mono- and di-ethanolamine. The quantitative methods are based on the conversion of the amines to their *p*-brombenzene sulfonyl derivatives. Color reactions and melting points of the derivatives may be used as tests for identity. Typical results are presented.

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### COLORIMETRIC DETERMINATION OF PILOCARPINE AND ITS SEPARATION FROM OTHER ALKALOIDS

By IRWIN S. SHUPE (Cosmetic Division,\* U. S. Food and Drug Administration, Federal Security Agency, Baltimore, Md.)

Pilocarpine and its salts have been used for some time in certain types of cosmetic hair preparations. The alkaloid has also been proposed for use in skin lotions.<sup>1</sup>

In hair lotions in particular, other alkaloids (such as quinine) are often used in conjunction with pilocarpine. Methods are suggested here for the separation of small quantities of pilocarpine from quinine and other alkaloids and for its subsequent colorimetric determination.

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\* D. Dahle, in charge.

<sup>1</sup> Drug and Cosmetic Review, 1940-41, 5th Ed., p. 307. Drug and Cosmetic Ind., N.Y.

## PRINCIPLES OF METHODS USED

Pilocarpine has been shown by Jowett<sup>2</sup> to contain a lactone group that can be saponified with alkali to yield a water-soluble salt. The alkaloid may be regenerated by treatment with acid. This property offers a basis for its separation from quinine and other alkaloids that do not react with fixed alkali. It was found possible to retain pilocarpine with a sodium hydroxide solution and to effect a complete removal of quinine and other alkaloids by extraction with chloroform. The pilocarpine can then be quantitatively recovered from the alkaline solution by acidification and extraction with chloroform from an ammoniacal solution.

The colorimetric procedure is based on a qualitative test first described by Helch<sup>3</sup> for the detection of pilocarpine hydrochloride. In a slightly acid solution, pilocarpine forms a blue compound in the presence of potassium dichromate and hydrogen peroxide that is soluble in benzene and chloroform. Both the U. S. Pharmacopoeia<sup>4</sup> and National Formulary<sup>5</sup> include this test for the identity of pilocarpine salts. Bredebach<sup>6</sup> characterizes the blue colored material as pilocarpine perchromate. It should not be confused, however, with the blue perchromic acids formed with peroxide and dichromate. The latter are insoluble in benzene and chloroform. The intensity of the blue color extracted by chloroform was found to be proportional to the quantity of pilocarpine present.

The comparison or measurement of the color intensities may be made by any standardized procedure. The color is sufficiently stable to be adapted to photometric instruments, such as the Clifford type<sup>7</sup> neutral wedge photometer. This instrument was used for the color measurements. The color shows a maximum absorption at 560 m $\mu$  wave length.

## METHODS

## STANDARD PILOCARPINE SOLUTION

Dissolve an accurately weighed portion of an assayed pilocarpine salt in water to make a solution containing ca. 0.2 mg. of pilocarpine per ml. The pilocarpine salt may be assayed by the A.O.A.C. method<sup>8</sup> or other suitable procedure.

## SEPARATION OF PILOCARPINE FROM QUININE (AND OTHER ALKALOIDS)

To 10 ml. of an aqueous solution of alkaloids containing pilocarpine, add ca. 0.1 gram of NaHSO<sub>3</sub> and a 3 ml. excess of 10% NaOH. Mix, and let stand 5 minutes. Extract with five 20 ml. portions of CHCl<sub>3</sub>. Wash the CHCl<sub>3</sub> extracts with 5 ml. of water. (The washed CHCl<sub>3</sub> extracts may be retained for the determination of quinine and other alkaloids.)

Add the 5 ml. of wash water to the residual alkaline solution and add 2 ml. excess of concentrated HCl. Mix, and let stand ca. 15 minutes. Add a slight excess of ammonia and extract *immediately* with five 20 ml. portions of CHCl<sub>3</sub>. Filter the

<sup>2</sup> J. Chem. Soc., 77, 473 (1900).

<sup>3</sup> Pharm Post., 35, 289 (1902).

<sup>4</sup> U. S. Pharmacopoeia XI, p. 290.

<sup>5</sup> National Formulary VI, p. 286.

<sup>6</sup> Apoth. Ztg., 49, 723 (1933).

<sup>7</sup> This Journal, 19, 130 (1936).

<sup>8</sup> Methods of Analysis, A.O.A.C., (1940), p. 589.

$\text{CHCl}_3$  through a pledget of cotton into a beaker. Evaporate the  $\text{CHCl}_3$  on a steam bath.

Reserve the evaporated residue of pilocarpine for colorimetric determination.

#### DETERMINATION OF PILOCARPINE

**Volumetric.**—To determine the approximate quantity of pilocarpine in the  $\text{CHCl}_3$  residue after separation from other alkaloids, dissolve the residue in water and make to 25 ml. in a volumetric flask. Titrate one 10 ml. portion with 0.02 *N* acid, using methyl red indicator. 1 ml. of 0.02 *N* acid = 4.16 mg. of pilocarpine.

**Colorimetric.**—To six 150 ml. separatory funnels, transfer 10 ml. (or smaller portion) of sample containing 0.2–2.0 mg. of pilocarpine and aliquots of 10, 5, 2.5, 1.0, and 0.5 ml. of standard pilocarpine solution. Add water if necessary to make to 10 ml. volume.

To each funnel add 1 ml. of the acetic acid and an accurately measured 20 ml. portion of  $\text{CHCl}_3$ . Then add 1 ml. each of 5%  $\text{K}_2\text{CrO}_4$  and 3%  $\text{H}_2\text{O}_2$ . Shake vigorously for ca.  $\frac{1}{2}$  minute. Allow the layers to separate and filter the  $\text{CHCl}_3$  through filter papers into small glass-stoppered flasks. Protect from direct sunlight and as much as possible from daylight (cf. Table 11).

Compare the colors, preferably in a photometer. If a visual, neutral-wedge photometer is used, take an average scale reading on each  $\text{CHCl}_3$  solution. Use a No. 56 filter and a 2 inch cell. (Other sizes of cells are optional for different ranges of pilocarpine.)

Plot the average scale readings against mg. of pilocarpine. From the scale reading on the sample, calculate its equivalent in pilocarpine.

#### EXPERIMENTAL ON EXTRACTION PROCEDURE

In order to determine the proper conditions necessary for the retention of pilocarpine in alkaline solution and its subsequent recovery, a study was made of the effect of the various reagents.

**Effect of sodium bisulfite.**—Although pilocarpine is relatively stable to oxidizing agents in acid solutions, some low recoveries in preliminary experiments indicated a loss through oxidation in the alkaline solution. The addition of sodium bisulfite prevented this loss. Table 1 illustrates the relative loss apparently caused by oxidation from air. The pilocarpine was allowed to stand 5 minutes in the presence of 2 per cent sodium hydroxide before acidification and extraction as directed in the method.

The colorimetric determinations indicated in the table were made with a neutral-wedge photometer. Volumetric titrations were made by the

TABLE 1.—Effect of  $\text{NaHSO}_3$  on recovery of pilocarpine from alkaline solution

EXP. NO.	PILOCARPINE IN 2% NaOH	$\text{NaHSO}_3$ ADDED	RECOVERY OF PILOCARPINE	
			COLORIMETRIC	VOLUMETRIC
	mg./10 ml.	grams	per cent	per cent
1	37.4	None	—	94
2	37.4	0.1	—	100
3	1.0	None	99	—
4	1.0	0.1	100	—

A.O.A.C. method,<sup>8</sup> methyl red being used as indicator. A minimum of indicator permits a better observance of the red color at the end point.

*Effect of sodium hydroxide concentration.*—To test for the complete retention of pilocarpine, a series of experiments was made with varying concentrations of sodium hydroxide. With less than 0.5 per cent sodium hydroxide present, pilocarpine was slowly but partially extracted with chloroform. An unduly large excess of alkali, on the other hand, may be expected to favor other decomposition of the alkaloid. Table 2 shows that the minimum quantity of sodium hydroxide required is about 2 per cent.

Although pilocarpine is readily hydrolyzed by strong aqueous alkalies, it shows an interesting stability in chloroform solutions. Such solutions of the alkaloid are not affected by shaking with 5 per cent aqueous sodium hydroxide. This offers a means of separating phenolic alkaloids, which will go into the aqueous alkaline layer.

TABLE 2.—*NaOH concentration required for detention of pilocarpine*

EXP. NO.	CONTAINED PILOCARPINE	NaOH CONCENTRATION	PILOCARPINE REMOVED BY ONE 25 ML. CHCl <sub>3</sub> EXTRACT
	mg./10 ml.	per cent	mg.
1	10	0.5	1.6
2	10	1.0	0.02
3	10	2.0	0.00
4	10	3.0	0.00
5	10	5.0	0.00
6	10	10.0	0.00

*Effect of acid concentration on regeneration of pilocarpine.*—The sodium salt of pilocarpine is not immediately converted to pilocarpine by treatment with acids. The regeneration of the lactone requires time and is dependent on the acid concentration. This is illustrated in Table 3.

TABLE 3.—*Effect of time and acid concentration on regeneration of pilocarpine from alkaline solution*

EXP. NO.	CONTAINED PILOCARPINE IN 2% NaOH	CONC. HCl ADDED IN EXCESS	TIME INTERVAL BEFORE ADDING EXCESS NH <sub>4</sub> OH AND EXTRACTION OF PILOCARPINE	PILOCARPINE RECOVERY (VOLUMETRIC)
	mg./10 ml.	ml.	minutes	per cent
1	10	0.5	Immediate	5
2	10	0.5	10	39
3	10	1.0	10	81
4	10	2.0	Immediate	18
5	10	2.0	10	100
6	10	2.0	15	100
7	10	5.0	Immediate	63
8	10	5.0	10	100

*Effect of excess ammonium hydroxide on recovery of pilocarpine.*—A high concentration of ammonium hydroxide may be expected to retain pilocarpine partially. The data in Table 4 show that the presence of strong ammonia does cause an apparent hydrolysis and results in incomplete recovery.

TABLE 4.—*Effect of  $\text{NH}_4\text{OH}$  concentration on recovery of pilocarpine*

EXP. NO.	CONTAINED PILOCARPINE IN 10 ML. $\text{H}_2\text{O}$	$\text{NH}_4\text{OH}$ ADDED	TIME OF STANDING IN PRESENCE OF ADDED AMMONIA*	RECOVERY OF PILOCARPINE (VOLUMETRIC)
	mg.	ml. per cent	minutes	per cent
1	37	1-10	Immediate	100
2	37	1-10	5	100
3	37	1-30	Immediate	100
4	37	1-30	5	90
5	37	5-30	Immediate	92

\* About 0.1 gram of  $\text{NaHSO}_3$  was added to prevent oxidative decomposition.

*Volatility of pilocarpine base.*—Aliquots of a chloroform solution of pilocarpine alkaloid were treated as shown in Table 5 to determine losses caused by heat. The results show that chloroform extracts may be evaporated to dryness on a steam bath without loss. Continued heating at  $100^\circ\text{C}$ . or the presence of moisture caused low recoveries.

TABLE 5.—*Volatility of pilocarpine base*

EXP. NO.	CONTAINED PILOCARPINE IN DRY $\text{CHCl}_3$	HEAT	RECOVERY OF PILOCARPINE
	mg.		per cent
1	1	None (spontaneous evap.)	100 (Colorimetric)
2	1	15 minutes on steam bath	100 (Colorimetric)
3	1	15 minutes $100^\circ\text{C}$ . oven	100 (Colorimetric)
4	25	None (spontaneous evap.)	100 (Volumetric)
5	25	15 minutes on steam bath	100 (Volumetric)
6	25	15 minutes on steam bath with 5 ml. $\text{H}_2\text{O}$ added	97 (Volumetric)
7	25	30 minutes $100^\circ\text{C}$ . oven	97 (Volumetric)

#### EXPERIMENTAL ON COLORIMETRIC DETERMINATION

In each series of experiments followed in studying the effect of a particular reagent, the other reagents were used as directed in the colorimetric method. These are listed as "constants" after each table. The color measurements were made with a neutral wedge photometer and a filter centered at about  $560\text{ m}\mu$ .

*Effect of acids on color development.*—Acidity appeared to have the



greatest influence on the color produced with pilocarpine and perchromic acids. Adjustment to the proper acidity with mineral acids (sulfuric, hydrochloric) required precise control since an excess destroyed the color. Acetic acid was found to be much more suitable. The results (Table 5) show that considerable excess of acetic acid may be used.

TABLE 6.—*Effect of acids on color development*

EXP. NO.	CONTAINED PILOCARPINE IN WATER	ACID ADDED	PHOTOMETER SCALE READING 2 IN. CELL
	<i>mg./10 ml.</i>	<i>ml.</i>	
1	1	0.5 10% acetic	5.4
2	1	1.0 10% acetic	5.4
3	1	2.0 10% acetic	5.4
4	1	1.0 10% HCl	0.5
5	1	1.0 10% H <sub>2</sub> SO <sub>4</sub>	0.3

Constants: 1 ml. 5% K<sub>2</sub>CrO<sub>4</sub>.  
1 ml. 3% H<sub>2</sub>O<sub>2</sub>.  
20 ml. CHCl<sub>3</sub>.

TABLE 7.—*Effect of H<sub>2</sub>O<sub>2</sub> concentration*

EXP. NO.	CONTAINED PILOCARPINE IN WATER	H <sub>2</sub> O <sub>2</sub> USED 3% SOLUTION	PHOTOMETER SCALE READING
	<i>mg./10 ml.</i>	<i>ml.</i>	
1	1	0.2	5.2
2	1	0.5	5.4
3	1	1.0	5.4
4	1	2.0	5.4

Constants: 1 ml. 10% acetic acid.  
1 ml. 5% K<sub>2</sub>CrO<sub>4</sub>.  
20 ml. CHCl<sub>3</sub>.

TABLE 8.—*Effect of K<sub>2</sub>CrO<sub>4</sub> concentration*

EXP. NO.	CONTAINED PILOCARPINE IN WATER	K <sub>2</sub> CrO <sub>4</sub> USED 5% SOLUTION	PHOTOMETER SCALE READING
	<i>mg./10 ml.</i>	<i>ml.</i>	
1	1	0.5	5.4
2	1	1.0	5.4
3	1	2.0	5.4

Constants: 1 ml. 10% acetic acid.  
1 ml. 3% H<sub>2</sub>O<sub>2</sub>.  
20 ml. CHCl<sub>3</sub>.

*Effect of hydrogen peroxide and potassium chromate.*—Varying the amounts of these two reagents had little influence on the color. Tables 7 and 8 show that plus 100 per cent or minus 50 per cent of the quantities designated in the method caused no variation in the readings.

*Effect of Salts.*—Ammonium chloride, sulfate, and nitrate were added to pilocarpine solutions to determine the influence of different acid radicals. The absence of any interference is shown in Table 9.

TABLE 9.—*Effect of salts*

EXP. NO.	CONTAINED PILOCARPINE IN WATER	SALT ADDED	PHOTOMETER SCALE READING
	mg./10 ml.		
1	1	None	5.4
2	1	100 mg. $\text{NH}_4\text{NO}_3$	5.4
3	1	100 mg. $\text{NH}_4\text{Cl}$	5.4
4	1	100 mg. $(\text{NH}_4)_2\text{SO}_4$	5.4

Constants: 1 ml. 10% acetic acid.  
 1 ml. 5%  $\text{K}_2\text{CrO}_4$ .  
 1 ml. 3%  $\text{H}_2\text{O}_2$ .  
 20 ml.  $\text{CHCl}_3$ .

*Effect of aqueous volume.*—Slight variations in the volume of aqueous solution from which the blue color is extracted caused no measurable change in the color intensity. Results of Table 10 indicate about 98 per cent of the color taken up in the 20 ml. chloroform layer.

TABLE 10.—*Effect of aqueous volume*

EXP. NO.	CONTAINED PILOCARPINE	AQUEOUS VOLUME BEFORE ADDITION OF REAGENTS	PHOTOMETER SCALE READING
	mg.	ml.	
1	1	5	5.45
2	1	8	5.40
3	1	10	5.40
4	1	12	5.40
5	1	15	5.35
6	1	20	5.30

Constants: 1 ml. 10% acetic.  
 1 ml. 3%  $\text{H}_2\text{O}_2$   
 1 ml. 5%  $\text{K}_2\text{CrO}_4$ .  
 20 ml.  $\text{CHCl}_3$ .

The partition ratio between a blue chloroform solution equivalent to 1 mg. of pilocarpine and the aqueous reagents was 99 to 1.

*Effect of light on stability of color.*—Colored chloroform solutions from 1 mg. portions of pilocarpine developed according to the method were al-

lowed to stand in glass-stoppered flasks. Readings were taken at the time intervals shown in Table 11, after exposure to various degrees of light.

TABLE 11.—*Effect of light on color*

LIGHT	SCALE READINGS AFTER TIME INTERVAL OF—				
	IMMEDIATE	15 MINUTES	30 MINUTES	1 HOUR	2 HOURS
Dark	5.4	5.4	5.4	5.4	5.4
Indirect diffused daylight	5.4	5.4	5.4	5.4	5.4
Direct sunlight	5.4	2.3	0.6	—	—

TABLE 12.—*Standard series*

PILOCARPINE	PHOTOMETER SCALE READING*
mg.	
0.0	0.10
0.125	0.75
0.25	1.45
0.50	2.75
0.75	4.10
1.00	5.40
1.50	8.15
2.00	10.70
2.50	13.2

\* With 2 inch cell and filter No. 56.

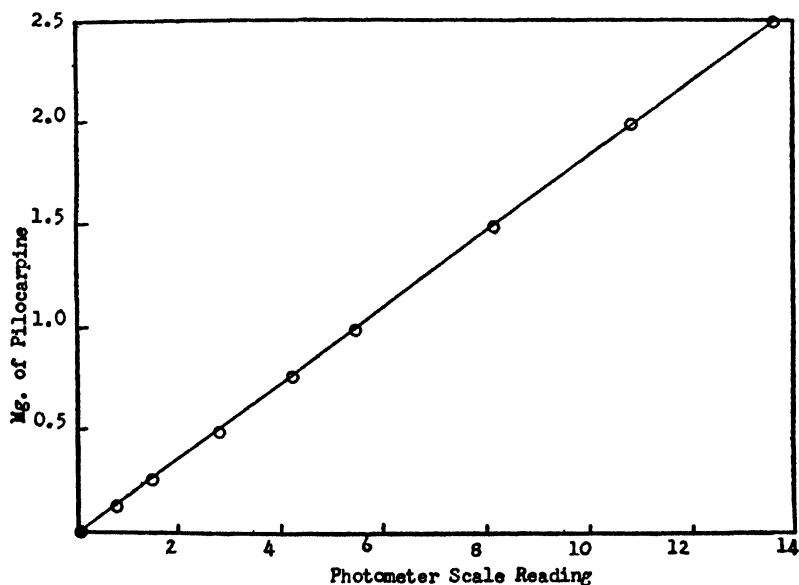


FIG. 1.

Although the colors are stable for 2 hours or longer, when protected from light, it is recommended that readings be made as soon as possible and uniformly after their development to avoid errors from temperature changes and evaporation.

*Standard series.*—The photometer scale readings shown in Table 12 and the corresponding graph, Figure 1, illustrate the type of curve obtained for a standard series. A standard pilocarpine solution was prepared from a specimen of pilocarpine nitrate that assayed 74.9 per cent alkaloid.

#### RECOVERY EXPERIMENTS

Data on recoveries of pilocarpine in the presence of quinine and some other alkaloids are shown in Table 13. Volumetric determinations of the pilocarpine residues were made with the larger quantities after separation by the colorimetric extraction procedure.

TABLE 13.—*Recoveries of pilocarpine from mixtures with other alkaloids*

EXP. NO.	CONTAINED IN 10 ML. OF AQUEOUS SOLUTION		PILOCARPINE RECOVERY	
			VOLUMETRIC	COLORIMETRIC
		mg.	per cent	per cent
1	Pilocarpine	74.8	98.1	—
2	Pilocarpine	37.4	98.6	—
3	Pilocarpine	10.0	—	99.0
4	Pilocarpine	1.0	—	98.2
5	Pilocarpine	1.0	—	97.6
	Quinine	100.0		
6	Pilocarpine	1.0	—	97.8
	Quinine	10.0		
7	Pilocarpine	10.0	97.0	98.2
	Quinine	10.0		
8	Pilocarpine	1.0	—	98.0
	Quinine	10.0		
	Quinidine	10.0		
	Cinchonine	10.0		
	Cinchonidine	10.0		
	Strychnine	10.0		
	Brucine	10.0		

#### SUMMARY

Methods suitable for the determination of pilocarpine in cosmetics have been described.

An extraction method, based on the retention of pilocarpine in aqueous alkaline solutions, is offered for the separation of pilocarpine from other alkaloids.

A modified Helch color test has been adapted to the colorimetric estimation of pilocarpine.

The effects of variations in the concentrations of the reagents have been studied.

Recovery data for mixtures with quinine and some other alkaloids are included.

### DETERMINATION OF HYDROXIDE IN BASIC COPPER SULFATES USED AS FUNGICIDES

By H. Bois (U. S. Department of Agriculture, Agricultural Marketing Service, San Francisco, Calif.)

Basic copper sulfates, when dissolved in an excess of dilute acids, form neutral copper salts. The alkalinity of such compounds can be determined by simple back titration after solution in an excess of acid if suitable precautions are taken to overcome the masking effect of the copper ion on the end-point color.

The titration of acidity in copper solutions, with methyl orange as an indicator, is a well-known procedure,<sup>1</sup> of which the following method is an adaptation:

Weigh into a 500 ml. Erlenmeyer flask a sample, the alkalinity of which is equivalent to 25–45 ml. of 0.2 *N* acid. Add ca. 125 ml. of water, heat to boiling, and keep gently boiling while adding 0.2 *N* H<sub>2</sub>SO<sub>4</sub> slowly from a buret. When solution is complete, remove mixture from flame, cool, and add water to bring the volume to ca. 300 ml. In another 500 ml. Erlenmeyer flask make up a copper solution that closely approximates in appearance, both as to color and volume, the solution in the first flask, by adding 10% CuSO<sub>4</sub> solution to water. Add 0.1% methyl orange indicator dropwise to the *second* flask until the blue color of the copper is completely masked (usually 3–7 drops are sufficient); then add the same quantity of indicator to the first flask and titrate the excess acidity with 0.1 *N* or 0.2 *N* NaOH to the methyl orange end point, using the color of the second flask as a guide. If the quantity of NaOH used is great, a corresponding quantity of water may be added to the second flask. From the two titrations calculate alkalinity as hydroxide.

### EXPERIMENTAL

The object of the experiments was to effect a comparison between the errors introduced by various quantities of copper with the quantities of acid required to bring these quantities of copper into solution.

Known quantities of acid were added to known quantities of copper sulfate solution, approximate blanks were prepared, and back titrations

<sup>1</sup> Bur. Standards Circ. No. 52.

were obtained to the methyl orange end point according to the directions given previously. Results are shown in the table. In calculating the values in the second and sixth columns, the copper is assumed to have been added as  $\text{CuSO}_4 \cdot 2 \text{Cu(OH)}_2$ .

The results follow:

Cu TAKEN	EQ. OF Cu IN 0.1 N $\text{H}_2\text{SO}_4$	0.1 N ACID ADDED	BACK TITRATION AS 0.1 N	ERROR	
grams	ml.	ml.	ml.	ml.	per cent
0.0835	17.51	43.35	43.33	0.02	0.11
0.2087	43.77	43.35	43.34	0.01	0.02
0.4175	87.54	43.35	43.23	0.12	0.14
0.8350	175.1	43.35	43.32*	0.03	0.02
2.087	437.7	43.35	42.42	0.93	0.21

\* Analysis made by Edward A. Lasher.

The results show that while the errors are in the same direction and tend to increase with the quantity of copper used, they are small when compared with the total titration in the case of a basic copper sulfate.

## ERRORS OF MUNSON AND WALKER'S REDUCING-SUGAR TABLES AND THE PRECISION OF THEIR METHOD

By RICHARD F. JACKSON and EMMA J. McDONALD (National Bureau of Standards, Washington, D. C.)

### I. INTRODUCTION

In recent years advances in reducing-sugar analysis have consisted mainly in improvements in volumetric processes, especially those that permit the entire analysis to be completed in a single reaction vessel. While these methods are of most general service, the older methods, which require filtration of cuprous oxide, still serve a useful purpose, particularly when but an occasional analysis is required or the sample is seriously contaminated or discolored. The gravimetric methods have the disadvantage of being more time-consuming than the volumetric processes but when modified by the introduction of volumetric methods for the determination of reduced copper they approach the volumetric methods with respect to convenience and rapidity.

Practically the only one of the older gravimetric methods that has survived in this country and that indeed is still extensively used at the present time is the method of Munson and Walker (1). This method has the advantages of extreme simplicity and, as will be shown, high precision.

Munson and Walker, in establishing their fundamental tables in 1906, analyzed the respective pure sugars, determining the reduced copper by weighing as cuprous oxide, which they then converted to copper by the stoichiometrical factor. Erb and Zerban (2) redetermined the copper values for sucrose-invert sugar mixtures containing 0.4 gram of total sugar. Their results are in agreement with those of Munson and Walker for the middle range of concentrations, but in disagreement at the higher concentrations.

Recently Hammond (3) made a comprehensive revision of the tables for dextrose, levulose, invert sugar, and three series of mixtures of sucrose and invert sugar containing respectively, 0.3, 0.4, and 2.0 grams of total sugar. Many of the copper values found by Hammond differ from those of Munson and Walker by amounts far greater than any probable experimental error. In view of the importance of these tables, it seemed advisable that a third series of analyses be made in order that one or the other sets of values might be corroborated. It was the purpose of the present investigation to contribute a third series and to determine, if possible, the source of the discrepancies between Hammond's and Munson and Walker's copper equivalents.

## II. METHODS OF ANALYSIS

### 1. MATERIALS AND REAGENTS

#### (a) *Soxhlet Reagent*

The Soxhlet reagent was prepared in the usual manner by mixing 25 ml. of each of the two constituent solutions immediately before the analysis. The copper solution contained 34.639 grams of copper sulfate crystals in 500 ml. of solution. The solution was allowed to stand for several days and was then filtered through fritted glass. The crystals were usually deficient in copper by 1 to 2 per cent and thus the copper content of the filtrate required adjustment of 440.9 mg. in 25 ml. For the median range of sugar concentrations it is apparently unimportant that the copper be adjusted accurately to the specified value, but at the higher concentrations, when the copper approaches exhaustion, it is important that the copper content conform to the specification.

The alkaline tartrate solution contained 173 grams of Rochelle salt and 50.0 grams of sodium hydroxide in 500 ml. of solution. The sodium hydroxide was freed from carbonate by allowing a 50 per cent solution to stand overnight and filtering through asbestos or fritted glass. The alkali content of the filtrate was determined by titration of weighed samples.

#### (b) *Sugars*

*Dextrose and levulose.*—The dextrose (Standard Sample No. 41 of this Bureau), which had been prepared by crystallization from aqueous solu-

tion in the anhydrous crystalline form, showed no loss in weight upon heating at 110° C. for 2 hours. The levulose, which had been prepared by crystallization from aqueous solution, was further purified by twice recrystallizing from aqueous alcohol (4). During the first recrystallization a volume of nitric acid stoichiometrically equivalent to the inorganic impurities was added. The final crystals, dried at 70° C., showed an ash content of less than 0.002 per cent and an absence of moisture when dried in a thin layer for 2 hours at 70° C.

*Invert sugar.*—For most of the analyses invert sugar was prepared by taking equal weights of pure dextrose and levulose. Hammond prepared his invert sugar in the same way, but his data, in spite of the high purity of the substance, showed considerably lower yields of copper than those of Munson and Walker, who prepared invert sugar by hydrolysis of sucrose with 0.02 *N* hydrochloric acid for "one-half hour on the water bath." It was thought at first that the differences in the observed reducing power might be due to differences in the invert sugar. An effort was accordingly made to reproduce Munson and Walker's measurements.

The velocity of inversion of cane sugar is in the highest degree a function of the temperature. Hence merely placing a solution on the water bath without regarding the temperature actually attained leads to uncertain results. In one instance such an experiment produced a preparation that was but 83 per cent inverted. Jackson and Gillis (5) derived formulas that enabled the writers to calculate the velocity of inversion as a function of temperature and concentration of hydrochloric acid. It was calculated that at 93° C. in the presence of 0.02 *N* acid, inversion was 99.99 per cent complete in 16 minutes.

A solution was prepared in an Erlenmeyer flask containing 1.9665 grams of sucrose, 78.2 ml. of water, and 20 ml. of 0.1 *N* hydrochloric acid. The position of the flask was so adjusted in the steam bath that the solution attained a temperature of 92° C. in about 7 minutes. It was allowed to remain in the bath for an additional 23 minutes, during which time the temperature remained between 92° and 94° C. The solution was cooled, neutralized carefully with sodium hydroxide (bromocresol green), and made up to a volume of 500 ml. The average of 5 analyses showed that 50 ml. of this solution containing 207 mg. of invert sugar precipitated 375.0 mg. of copper. On the other hand, 207 mg. of pure synthetic invert sugar precipitated 375.6, as shown by the mean of 4 determinations. Evidently Munson and Walker's high values for invert sugar are not due to the greater reducing power of hydrolyzed sucrose. The explanation of their excessive precipitations will be discussed in a later paragraph.

The deficiency in reducing power of sucrose hydrolyzed at a high temperature is in harmony with the measurements of the rotatory powers of invert sugar prepared by hydrolysis of sucrose (6) made by the writers, who endeavored to show that the rotation of invert sugar is a function of



the temperature at which the inversion is carried out, and the higher the temperature of inversion the greater the decomposition of invert sugar, even when the time of inversion is carefully chosen to avoid decomposition of the reaction products. Hence, to prepare a standard invert sugar solution from sucrose elevated temperatures of inversion must be avoided.

Table 1 shows the reducing power of invert sugar prepared under various conditions of inversion. Each solution was prepared by inverting 1.9665 grams of sucrose and making up to a final volume of 500 ml. A 50 ml. aliquot contained 207 mg. of invert sugar. The acid in each preparation was neutralized with sodium hydroxide, yielding small amounts of sodium chloride. Parallel experiments with mixtures of invert sugar and as much as 500 mg. of sodium chloride showed that the salt had a negligible influence on the weight of copper reduced. As appears from the tabulated values, standard invert sugar solutions having the same reducing power as pure synthetic invert sugar can be prepared by room-temperature inversion. If the inversion is carried out at higher temperatures a slightly diminished reducing power results.

TABLE 1.—*Reducing power of 207 mg. of invert sugar prepared from sucrose*

TEMPERATURE OF INVERSION	ACIDITY	TIME OF INVERSION	VOLUME OF SOLUTION CONTAINING 1.9665 g. OF SUCROSE	COPPER REDUCED
°C.		hours	ml.	mg.
93	0.02 <i>N</i> HCl	0.5	100	375.0
76	.01 <i>N</i> HCl	3.2	11	374.8
55	.1 <i>N</i> HCl	4	11	374.9
24	.65 <i>N</i> HCl	70	100	375.5
23	1.0 <i>N</i> H <sub>2</sub> SO <sub>4</sub>	50	50	375.7
Synthetic invert sugar				375.6

For the inversion at laboratory temperature the procedure of Lane and Eynon (7) is suitable. "A solution of 9.5 grams of pure sucrose is treated with 5 ml. of hydrochloric acid (sp. gr. 1.19) made up to about 100 ml., left at room temperature for about a week at 12 to 15° or 3 days at 20° to 25° C. and then made up to 1 liter. A known volume of the standard solution is neutralized with sodium hydroxide and suitably diluted immediately prior to use."

In the present series 3.993 grams of sucrose was dissolved in 87.5 ml. of water and acidified with 10 ml. of 6.34 *N* hydrochloric acid (Clerget acid). The mixture was allowed to stand at 23.5°–24.5° C. for 71 hours and was then neutralized with sodium hydroxide and made to a volume of 1 liter. Fifty milliliters of this solution contained 207 mg. of invert sugar and 185 mg. of sodium chloride.

## 2. DETERMINATION OF REDUCED COPPER

## (a) Cuprous Oxide Method

Munson and Walker determined the precipitated copper by directly weighing the cuprous oxide and converting to metallic copper by the stoichiometrical factor. They stated that the method had been checked against the electrolytic and the thiosulfate methods and the accuracy of the cuprous oxide procedure demonstrated. The applicability of this stoichiometrical factor has been extensively debated in subsequent literature and apparently agreement has been reached that, while cuprous oxide is a true measure of copper in the analysis of pure sugars, it is unreliable for the analysis of crude substances because of the likelihood of contamination by impurities derived from the substance undergoing analysis. This was demonstrated by Sherwood and Wiley (8) in an extended series of analyses.

In an effort to explain the source of the considerable discrepancies between Hammond's and Munson and Walker's copper values the writers duplicated Munson and Walker's cuprous oxide procedure in detail, and in addition determined the true reduced copper by thiosulfate titration or by electrolysis.

There was available a series of platinum Gooch crucibles that had very fine perforations and held the asbestos with but negligible loss during analysis. The asbestos was prepared by the method of Brewster and Phelps (9). The analytical results are shown in Table 2, each figure in the table representing the mean of at least four analyses. In every instance but one the copper calculated from the cuprous oxide is higher than the true copper as shown by thiosulfate titration or electrolysis. Thus even with pure sugars the cuprous oxide is contaminated with organic decomposition products.

TABLE 2.—Contamination of cuprous oxide

WEIGHT OF SUGAR	CUPROUS OXIDE	COPPER		
		BY FACTOR	BY ELECTROLYSIS	BY THIOSULFATE
mg.	mg.	mg.	mg.	mg.
207 (Invert, synthetic)	423.8	376.4	376.0	375.7
207 (Invert, sucrose)	424.7	377.2	—	375.5
117 (Dextrose)	258.5	229.6	—	229.7
207 (Dextrose)	438.1	389.1	388.1	388.0
207 (Levulose)	412.3	366.3	364.5	—
207 (Invert, plus 1.793 g. of sucrose)	439.3	390.2	385.7	385.7

The cuprous oxide from 207 mg. of invert sugar prepared from sucrose shows a contamination of 1.7 mg. (Table 2). The difference between Hammond's and Munson and Walker's copper is 1.8 mg. The cuprous

oxide from 2.0 grams of total sugar containing 207 mg. of invert sugar has a contamination of 4.5 mg., while Hammond differs from Munson and Walker by 4.7 mg. A similar comparison of the dextrose values fails because Hammond obtained more copper than did Munson and Walker. The average contamination of all the cuprous oxide precipitates in Table 2 is 1.6 mg., while the average difference between Hammond and Munson and Walker in 46 analyses is 1.8 mg. In other words, it appears highly probable that the entire difference between the two series of analyses is due to the fact that Munson and Walker's cuprous oxide was contaminated by appreciable quantities of organic decomposition products.

It is of interest to inquire why a so easily demonstrable contamination has not been revealed before. It is doubtless due to the fact that in most instances the comparison has been made with dextrose, which alone among the sugars causes a negligible contamination, at least at median concentrations of sugar. Thus up to a concentration of 180 mg. of sugar Hammond differs from Munson and Walker by an average of only 0.2 mg. of copper. In Table 2, 117 mg. of dextrose produced an uncontaminated precipitate, but the contamination became appreciable at 207 mg. of sugar.

The conclusion seems justified that Munson and Walker's tables, insofar as they relate sugar to metallic copper, are in error by the amount of the contamination. The apparent cuprous oxide-sugar equivalents are probably substantially correct if the amount of contamination is a constant quantity. This constant relation will hold in general for relatively pure sugars, but will fail for crude substances. On the other hand, Hammond's electrolytic copper is uncontaminated, but he has introduced into his tables the calculated weights of cuprous oxide. These latter values lead to erroneous results when cuprous oxide is weighed for the very reason that they are not contaminated.

It seems highly advisable that all reducing-sugar analyses be referred to metallic copper. Therefore in the following paragraphs the writers have given attention to the analytical methods for the estimation of copper.

#### (b) *Thiosulfate Method*

The iodometric determination of copper, when conducted under conditions insuring complete reduction of copper, is capable of an accuracy comparable to that of the best volumetric methods. The reaction on which the method depends, namely  $2 \text{CuI}_2 \rightarrow 2 \text{CuI} + \text{I}_2$ , is reversible, but if precautions are taken to remove cuprous ions as completely as possible and to maintain a sufficient concentration of iodide ions, the reaction runs quantitatively from left to right within the errors of measurement. Under suitable conditions the reaction can be made to run quantitatively from right to left. Shaffer and Hartmann (10) have shown that this, the cuprous titration, can be applied directly to the Munson and Walker method, but

such a procedure causes a divergence from the specified routine of the method and will not be considered here.

Shaffer and Hartmann (10) studied the equilibria involved and showed that for amounts of copper up to 318 mg. per 100 ml. the final concentration of potassium iodide must be 4.2 grams (or more) in 100 ml. at the end of the titration. For greater amounts of copper the concentration of potassium iodide must be greater in direct proportion.

In the analyses reported here the volume of the copper solution was so adjusted by previous calculation and marking the Erlenmeyer flask that the volumes of the potassium iodide solution, thiosulfate, starch, and washings made the proper final concentration of potassium iodide. For the lowest concentration of copper the final concentration of potassium iodide was 2.1 grams in 50 ml.; for the highest (425 mg.), 6.7 grams in 115 ml.

Foote and Vance (11) have shown that the sharpness of the end point and the precision of analysis are enhanced by the addition of ammonium thiocyanate at the approximate end point of the thiosulfate titration. Since cuprous thiocyanate is more insoluble than cuprous iodide, the thiocyanate has the effect of more completely removing cuprous ions from the solution and thus furthering the reaction from left to right. Another important result of the addition of thiocyanate is that apparently the surface portions of the precipitated cuprous iodide are converted to cuprous thiocyanate and the small quantity of adsorbed iodine is released to react with the thiosulfate. At the end point the precipitate is white, whereas without the thiocyanate it is slightly purple.

Although, as shown by Whitehead and Miller (12), the effect of low concentrations of strong acids is not considerable, that of acetic acid is even less. In the experience of the writers the reliability of the analysis is increased when the titration is carried out in the presence of acetic acid.

In reducing-sugar analysis the cuprous oxide is dissolved in 5 ml. of 1+1 nitric acid. It has been the practice to neutralize the excess of nitric acid with sodium hydroxide and then add a few drops of acetic acid. This procedure is tedious and, as will be shown, unnecessary. The same object could be accomplished by adding a sodium acetate solution if the acetic acid that is released has no appreciable effect on the titer. A 1+1 solution of nitric acid is about 8.3 *N*. Five milliliters of this solution would be completely buffered by 10 ml. of a 4.22 *N* solution of sodium acetate. A maximum of 2.4 ml. of acetic acid is released by the prescribed volumes of nitric acid and sodium acetate. The data showing that the effect of this procedure is unappreciable are given in Table 3. The results must be considered merely comparative because the same figures are used to standardize the thiosulfate.

Of a pure copper sulfate solution 50 ml. portions were titrated with 0.1573 *N* thiosulfate (1 ml. = 10 mg. of Cu) to a final volume of about 100

ml., 2 grams of thiocyanate being added near the end of the titration.

TABLE 3.—*Effect of acetic acid on the thiosulfate titer*

REAGENT	NUMBER OF ANALYSES	COPPER
		mg.
0.1 ml. of acetic	7	318.8
2 ml. of acetic	5	318.8
5 ml. of acetic	1	318.7
5 ml. of 1+1 nitric	3	318.7
10 ml. of sodium acetate } <sup>a</sup>		

<sup>a</sup> This mixture releases 2.37 ml. of glacial acetic acid.

In order to assure that the ratio of thiosulfate to copper was constant with both large and small amounts of copper, a series of analyses of a copper sulfate solution was made. The results are shown in Table 4. To each solution were added 5 ml. of 1+1 nitric acid and 6 ml. of 8 *N* ammonium acetate. The analyses showing the greatest departure from the mean, namely the second, third, and fifth, were in error by 0.01, 0.01, and 0.02 ml., respectively.

TABLE 4.—*Constancy of the ratio of thiosulfate to copper covering the range of volumes used in the determinations*

VOLUME OF COPPER SULFATE	VOLUME OF FINAL SOLUTION	WEIGHT OF POTASSIUM IODIDE	TITER	RATIO: THIOSULFATE TO COPPER SULFATE
ml.	ml.	grams	ml.	
15.026	54	2.2	12.634	0.8408
20.094	64	2.6	16.884	.8403
25.042	74	3.0	21.070	.8414
33.895	93	3.4	28.502	.8409
45.455	116	4.8	38.244	.8414
50.004	127	5.7	42.039	.8407
58.937	144	6.3	49.562	.8409
Average	—	—	—	0.8409

*Procedure.*—Collect the reduced copper on a Gooch crucible and wash the beaker and precipitate free from unreduced copper. From a 5 ml. pipet add to the reaction beaker a few drops of 1+1  $\text{HNO}_3$  and drop the remainder carefully on the precipitate, covering the crucible closely with a watch-glass. Rinse the pipet and watch-glass, catching the rinsings in the beaker. Allow the  $\text{Cu}(\text{NO}_3)_2$  and washings from the beaker to drain into an Erlenmeyer flask. Remove the nitrogen oxides by prolonged digestion on the steam bath or follow the usual procedure, using bromine water (13).

Cool, and add 10 ml. of sodium acetate solution (574 grams of trihydrate per liter). Add a volume of KI solution (40 grams per 100 ml.) such that at the end of the titration the concentration of KI shall be 4.0–4.5 grams per 100 ml. (The KI should be added slowly and with continuous agitation.)

Titrate with 0.1573 *N* thiosulfate, preferably adding the solution at the rate of about 13 ml. per minute, Add a starch solution when the iodine color approaches disappearance and continue the titration until the blue starch iodide is just decolorized. Add ca. 2 grams of  $\text{NH}_4\text{SCN}$  and agitate until the salt is completely dissolved. Continue the titration to the disappearance of the blue color.

Standardize the thiosulfate (39 grams of crystals per liter = 0.1573 *N*) by titration against 0.2–0.4 gram of pure copper dissolved in 5 ml. of  $\text{HNO}_3$  (1+1) and treated as directed for the cuprous oxide or against a measured volume of a  $\text{CuSO}_4$  solution that has been analyzed by electrolysis. In the latter case add 2 ml. of acetic acid before titration. 1 ml. of thiosulfate should be equivalent to approximately 10 mg. of copper.

### (c) *Permanganate Method*

The original method of determining cuprous oxide by means of permanganate was devised by Mohr (14) who dissolved the precipitate in acidified ferric sulfate and titrated the resulting ferrous iron with permanganate that had been standardized against sodium oxalate. It was eventually discovered that the results were from 1–2 per cent too low. Schoorl and Regenbogen (15) modified the method by dissolving the cuprous oxide in neutral ferric sulfate, subsequently acidifying and titrating. The modification has apparently eliminated the errors of the original method. The analytical results here reported substantiate this conclusion.

In this investigation the method of Schoorl and Regenbogen was used with but one essential modification. For the purpose of oxidizing reducing impurities in the stock ferric sulfate solution, the previous procedure has been to treat the neutral solution with an amount of permanganate determined by titration of an acidified sample. This seems a doubtful expedient because, as is well known, permanganate behaves differently in acid and alkaline (or neutral) solution. The procedure now recommended is to titrate 50 ml. of the ferric sulfate solution acidified exactly as in the analytical process, and to use the determined titer as a zero-point correction. This has the added advantage that the small excess of permanganate that is required to produce the color change, and in general will be approximately the same in the analysis, is included in the correction.

*Standardization.*—(16) Prepare a solution, approximately 0.1573 *N*, containing 4.98 grams of  $\text{KMnO}_4$  per liter. After several days' aging, filter through asbestos or fritted glass.

Transfer 0.35 gram of pure  $\text{Na}_2\text{C}_2\text{O}_4$  (dried at  $103^\circ\text{C}.$ ) to a 600 ml. beaker. Add 250 ml. of  $\text{H}_2\text{SO}_4$  (1+19) previously boiled for 10 minutes and cooled to  $27^\circ\text{C} \pm 3^\circ$ . Stir until the oxalate is dissolved. Add 29–30 ml. of permanganate at a rate of 25–35 ml. per minute while stirring slowly. Allow the mixture to stand until the pink color disappears (ca. 45 seconds). Heat to  $55^\circ$  or  $60^\circ\text{C}.$  and complete the titration by adding permanganate dropwise until a faint pink color persists for 30 seconds. Allow each drop to become decolorized before adding the next.

Determine the excess of solution (usually 0.03–0.05 ml.) required to impart the same pink color to the same volume of acid boiled and cooled to  $55^\circ$ – $60^\circ\text{C}.$

Weight in grams of oxalate  $\times 948.7 \div \text{titer} = \text{mg. of copper per ml.}$

An equally satisfactory standard is arsenious oxide (17).

**Ferric sulfate.**—Dissolve 135 grams of ferric ammonium alum or 55 grams of  $\text{Fe}_2(\text{SO}_4)_3$  (anhydrous) and dilute to 1 liter. The  $\text{Fe}_2(\text{SO}_4)_3$  dissolves very slowly. Determine  $\text{Fe}_2(\text{SO}_4)_3$  in the stock supply by strong ignition to  $\text{Fe}_2\text{O}_3$ . Acidify 50 ml. with 20 ml. of 4 *N*  $\text{H}_2\text{SO}_4$  and titrate with permanganate to the slightest perceptible color change. Apply the titer (usually 0.03–0.08 ml.) as a zero-point correction in analytical titrations.

**Determination.**—(13) Filter the  $\text{Cu}_2\text{O}$  on a Gooch crucible and wash the beaker and precipitate thoroughly. Transfer the asbestos film to the beaker with the aid of a glass rod. Add 50 ml. of the  $\text{Fe}_2(\text{SO}_4)_3$  solution and stir vigorously until the  $\text{Cu}_2\text{O}$  is completely dissolved. Examine for complete solution, holding the beaker above the level of the eye. Add 20 ml. of 4 *N*  $\text{H}_2\text{SO}_4$  and titrate with standard permanganate to the same color change as in the titration for zero-point correction.

The end point is rendered sharper by the addition of one drop of ferrous phenanthroline indicator (0.7425 gram of orthophenanthroline monohydrate in 25 ml. of 0.025 *M*  $\text{FeSO}_4$ ).

TABLE 5.—Comparative analyses by permanganate and thiosulfate

SUBSTANCE	COPPER BY PERMAN- GANATE	NUMBER OF ANALYSES	DIFFERENCE BETWEEN EXTREMES	COPPER BY THIO- SULFATE	NUMBER OF ANALYSES	DIFFERENCE BETWEEN EXTREMES	DIFFERENCE, PERMAN- GANATE MINUS THIOSULFATE
mg.	mg.		mg.	mg.		mg.	mg.
69 Invert	133.12	4	0.89	133.29	5	0.67	−0.17
220 Invert	396.82	3	.39	397.08	3	.51	−.28
184 Dextrose	349.62	4	.70	349.35	4	.69	+ .27

**Discussion.**—The permanganate method as modified by Schoorl and Regenbogen yields highly accurate results. It is eminently suitable for small amounts of cuprous oxide and has indeed found its greatest serviceability for such small amounts that the titration can be carried out with 0.033 *N* permanganate. When larger amounts of cuprous oxide (200–400 mg.) are precipitated, great difficulty is encountered in dissolving the precipitate. In many of the analyses reported here a period of 45 minutes was required before the cuprous oxide was dissolved. Table 5 shows some of the analytical results obtained.

#### (d) Dichromate Method

In a previous paper Jackson and Mathews (4) described a method by which cuprous oxide was dissolved in an excess of acidified potassium dichromate and titrated back to an electrometric end point with ferrous sulfate. Since the advent of the indicator, orthophenanthroline (18), the same titration can be made colorimetrically. Some modification of the method is necessary because, whereas the electrometric titration can be made in 1.2 *N* acid, the colorimetric procedure requires that the final solution be about twice normal in hydrochloric acid. Below this acid concentration the end point is uncertain and slow of attainment.

The colorimetric and electrometric end points do not occur at exactly the same titer of ferrous sulfate. On back titration the color change occurs first and the large potential change when an additional 0.02 ml. (in average) of 0.1573 *N* ferrous sulfate has been added.

All of the earlier electrometric measurements were made with an instrument constructed in the laboratory similar to the one described by Forbes and Bartlett (19). The later measurements were made with a Serfass Electron-Ray Titrimeter (20). Comparative analyses showed that the earlier instrument had served satisfactorily.

The dichromate method is the most expeditious for determining copper. The cuprous oxide dissolves readily in the hydrochloric acid-dichromate solution, in contrast to the difficulty of dissolving in ferric sulfate. In point of precision it approaches the thiosulfate method very closely at the median and lower concentrations of sugar. At the high concentrations of sugar the results are from 0.1 to 0.2 per cent lower. The method would appear particularly serviceable when a considerable number of analyses are required quickly.

*Reagents.*—*Standard dichromate solution.*—0.1573 *N* (containing 7.7135 grams of crystals of pure  $K_2Cr_2O_7$  dried at 150° C. in 1 liter). 1 ml. of this solution = 10 mg. of copper.

*Hydrochloric acid.*—Ca. 6 *N*.

*Ferrous ammonium sulfate solution.*—61.9 grams of the hexahydrate and 5 ml. of concentrated  $H_2SO_4$  in 1 liter.

*Phenanthroline-ferrous complex.*—Dissolve 0.7425 gram of orthophenanthroline monohydrate in 25 ml. of 0.025 *M*  $FeSO_4$  solution (6.95 grams of  $FeSO_4 \cdot 7H_2O$  in 1 liter).

*Procedure.*—Estimate the volumes of  $K_2Cr_2O_7$ ,  $FeSO_4$ ,  $HCl$  and water that will give an assured excess of dichromate and yield a concentration of ca. 2 *N* acid in ca. 200 ml. of final volume. An error of 5% or in most cases even 10% in acid concentration, can be tolerated. Fill graduated cylinders with the required volumes of water and acid.

Collect the precipitated  $Cu_2O$  on a Gooch crucible and wash thoroughly. Detach the mat with a glass rod and transfer to the reaction beaker. Add a small volume of water and disintegrate the mat. Pipet accurately a volume of the standard dichromate in excess of the quantity required to oxidize the  $Cu_2O$ . In general the approximate weight of copper will be known or can be roughly estimated, but in any case a sufficient volume must be added to supply an assured excess. Add rapidly the whole required volume of hydrochloric acid with continuous stirring and continue to stir until all the  $Cu_2O$  is dissolved. Immerse the crucible in the solution and be assured that the adhering  $Cu_2O$  is dissolved. Remove the crucible with the glass rod and wash it with the water from the graduate. Add 1 drop of the phenanthroline solution and titrate with  $FeSO_4$  to the permanent appearance of the brown ferrous-phenanthroline complex. As the end point is approached the brown color appears and fades as each of the last few drops is added and the  $FeSO_4$  must be added until the color is permanent, the additions finally being in fractions of drops.

Determine the ratio of concentrations of  $FeSO_4$  and dichromate and from this ratio compute the volume of dichromate required for the oxidation of  $CuO$ . This volume multiplied by 10 gives directly the mg. of copper reduced.

The titration can be conducted electrometrically if desired, in which case the ad-



dition of the indicator is unnecessary. Both colorimetric and electrometric end points can be determined in the same solution if desired.

### 3. THE REDUCTION REACTION

The reduction reaction was conducted in rigorous accordance with Munson and Walker's specifications. The analyses were made in 400 ml. beakers covered with watch-glasses. The solutions were brought to the boiling point in about 4 minutes by a previous adjustment of conditions. When the solution approached the boiling point there usually occurred a few sporadic periods of apparent boiling, which were followed by a very definite moment when the whole solution suddenly started to boil vigorously. This latter appearance was taken as the starting time of the 2-minute period. It was found important that the beakers after use be allowed to dry on a rack for at least one day and preferably several days. If they are thoroughly aerated in this way the time of boiling can be definitely recognized and no superheating or bumping occurs.

Since at the expiration of the 2-minute period of boiling the solutions remain approximately at the boiling temperature, it is important that the time required for filtration be uniform. By adjusting the vacuum and the thickness of the asbestos mat the rate of filtration was roughly controlled so that 30-50 seconds elapsed between the end of the boiling period and the completion of the filtration. Naturally in many analyses the time varied from this standard, and such variations probably contributed to the error of analysis, but in most instances imperceptibly.

Blank determinations were made of the amount of copper precipitated by boiling the mixed reagents without addition of sugar and collecting the precipitate on a Gooch crucible. This was moistened with 1+1 nitric acid, washed into a flask, and evaporated to dryness. The residue was dissolved in 0.25 ml. of acetic acid, transferred to a test tube, and made up to 5 ml. with water. A drop of potassium ferrocyanide was added, and the sample was compared colorimetrically with standards containing known weights of copper. Quite uniformly throughout the investigation this blank test showed 0.3 mg. of copper with maximum deviations of less than 0.05 mg. Munson and Walker (1) determined the copper reduced in blank experiments by weighing cuprous oxide. The average value from 67 determinations was 0.24 mg. of copper. They obtained many blanks of negative value. If these are rejected, together with the equally improbable ones of 1 mg. or more, their average is 0.33 mg. Both of these averages are in essential agreement with the values found in the analyses made by the writers.

The reduction of copper by the reagents is caused by the oxidizing effect of cupric copper on the alkaline tartrate. The extent of reduction probably varies with the concentration of copper and would not be the same if much of the copper is rapidly reduced by sugar. It therefore seems

a questionable procedure to apply a uniform blank correction to all analyses regardless of the concentration of sugar. Moreover, the analyst that uses the empirical tables seldom determines or applies a blank correction, and for his purpose the uncorrected tables are more directly applicable. For these reasons the writers shall in the following pages report the weights of copper precipitated by sugar exactly as determined without application of a blank correction. This procedure is in harmony with that of Hammond, who published the copper values obtained without correction for blanks.

The foregoing statements apply to Soxhlet reagents that are not more than a few months old. After long standing the blank becomes increasingly greater. If the analyst determines a blank correction and finds a value different from 0.3 mg., he has merely to deduct all but 0.3 from his copper precipitate in order to be consistent with Hammond's or the data presented in this paper.

### III. EXPERIMENTAL RESULTS

By the methods that have been described analyses were made of the three sugars, dextrose, levulose, and invert sugar at ten concentrations ranging from 23 to 230 mg. in 50 ml. of solution. At each concentration usually 8 determinations were made, in 4 of which the reduced copper was determined by thiosulfate titration and in the remaining 4 by colorimetric dichromate titration.

The results of the analyses of dextrose solutions are shown in Table 6. The experimental results for the thiosulfate analysis of copper (Column 2) were correlated by the method of least squares, the computation yielding the formula:

$$\text{Cu (by thiosulfate)} = 2.0820 d - 0.001005 d^2, \quad (1)$$

in which copper and dextrose ( $d$ ) are expressed in milligrams. The residuals (Column 4) are satisfactorily small.

It is of interest to compare this series of analyses with a series completed and published (21) previously, in which the copper was related to the dextrose by the formula:

$$\text{Cu (by thiosulfate)} = 2.0800 d - 0.000989 d^2. \quad (2)$$

The two independent series show no deviation (Column 6) as great as one part in a thousand. For tabulation the mean of these two formulas will be taken as the best result of this investigation.

$$\text{Cu (by thiosulfate)} = 2.0810 d - 0.000997 d^2. \quad (3)$$

In the last four columns of Table 6 are shown the results of the analysis by colorimetric dichromate titration. A least-square computation yielded the formula:

$$\text{Cu (by dichromate)} = 2.0792 d - 0.001005 d^2. \quad (4)$$

TABLE 6.—*Milligrams of copper reduced by dextrose*

WEIGHT OF DEXTROSE	COPPER BY THIOSULFATE					COPPER FROM HAMMOND'S TABLE	DIFFERENCE OF MEAN <sup>c</sup> FROM HAMMOND'S TABLE		COPPER BY DICHROMATE (COLORIMETRIC)			
	SERIES II			CALCU- LATED <sup>b</sup> FROM SERIES I	FOUND				CALCU- LATED <sup>d</sup>	FOUND MINUS CALCU- LATED	DICHROMATE MINUS THIOSULFATE (CALCULATED VALUES)	
	FOUND	CALCU- LATED <sup>a</sup>	FOUND MINUS CALCU- LATED									
mg.	46.3	47.3	-1.0	47.3	0	47.8	mg.	per cent	46.8	47.3	-0.5	0.0
23	94.0	93.7	+0.3	93.6	+0.1	94.0	-0.4	1.0	94.3	93.5	+0.8	-0.1
46	138.6	138.9	-0.3	138.8	+0.1	139.0	-0.2	0.4	138.3	138.7	-0.4	-0.1
69	183.2	183.0	+0.2	183.0	0	183.0	0	0.1	182.7	182.8	-0.1	-0.2
92	225.9	226.1	-0.2	226.1	0	225.8	+0.3	0	225.6	225.8	-0.2	-0.3
115	268.0	268.2	-0.2	268.2	0	267.6	+0.6	0.1	268.0	267.8	+0.2	-0.4
138	309.3	309.2	+0.1	309.2	0	308.5	+0.7	0.2	308.5	308.7	-0.2	-0.5
161	349.1	349.1	0	349.2	-0.1	348.3	+0.8	0.2	348.9	348.5	+0.4	-0.7
184	388.3	387.9	+0.4	388.2	-0.3	387.2	+0.8	0.2	387.2	387.3	-0.1	-0.9
207	425.4	425.7	-0.3	426.1	-0.4	424.8	+1.1	0.2	425.0	425.0	0	-1.1
230								0.3				

<sup>a</sup> Formula (1) Cu (by thiosulfate) = 2 0820 d - 0 001005 d<sup>2</sup><sup>b</sup> Formula (2) Cu (by thiosulfate) = 2 0800 d - 0 000989 d<sup>2</sup><sup>c</sup> Formula (3) Cu (by thiosulfate) = 2 0810 d - 0 000997 d<sup>2</sup> (mean of (1) and (2)).<sup>d</sup> Formula (4) Cu (by dichromate) = 2 0792 d - 0 001005 d<sup>2</sup>

In the low and medium ranges of concentration the dichromate titrations agree essentially with the thiosulfate values for total copper, but in the higher concentrations the dichromate values fall slightly below those by thiosulfate, the discrepancy rising to a maximum of 0.26 per cent. This difference occurs not only in the case of dextrose but also, as will appear below, in the cases of levulose and invert sugar. It cannot therefore be ascribed to analytical error, but must be explained by some more fundamental property of the reaction, which was not further investigated.

In the paper previously cited (21) a series of dichromate-dextrose equivalents was published. These are not directly comparable with those given in Table 6 because they were determined by electrometric titration at a hydrochloric-acid acidity of 1.2–1.4 *N*. Systematic experimentation showed a consistent difference of 0.06 ml. of dichromate between the electrometric end point in the presence of 1.2 *N* hydrochloric acid and the colorimetric end point in 2 *N* hydrochloric acid, whereas the mean experimental difference between the two respective series of sugar analyses proved to be .054, the electrometric titration giving in both cases the lower volume of dichromate. The two series of analyses thus yielded consistent results.

In Table 7 are assembled the data on the reducing action of levulose. Least-square computations yielded the respective formulas:

$$\text{Cu (by thiosulfate)} = 1.8818 \text{ l} - 0.000596 \text{ l}^2 \quad (5)$$

$$\text{Cu (by dichromate)} = 1.8840 \text{ l} - 0.000614 \text{ l}^2. \quad (6)$$

The small values of the residuals show that the formulas represent the data satisfactorily.

The reduction data on invert sugar are given in Table 8. Least-square adjustment yielded the formulas:

$$\text{Cu (by thiosulfate)} = 1.9834 \text{ i} - 0.000818 \text{ i}^2 \quad (7)$$

$$\text{Cu (by dichromate)} = 1.9828 \text{ i} - 0.000827 \text{ i}^2. \quad (8)$$

While the measurements were in progress it was expected that the rule of mixtures would apply to the problem and that the copper reduced by invert sugar would be the mean of the amounts precipitated by dextrose and by levulose. Such proved not to be the case. The copper reduced by invert sugar is less than the mean by the two constituents (with the exception of the lower concentrations), the deficiency rising to somewhat more than one-tenth per cent at the higher concentrations in the analyses by thiosulfate and to more than two-tenths per cent in those by dichromate.

The amount of copper reduced is a function not only of the concentration of sugar but also of the concentration of copper. If one constituent of a sugar mixture reacts upon the copper more rapidly than the other it will diminish the copper concentration before the second constituent has reacted. The latter then will undergo reaction in a lower concentration of copper when in a mixture than when alone, and theoretically should re-

TABLE 7.—Milligrams of copper reduced by levulose

WEIGHT OF LEVULOSE	COPPER BY THIOCYANATE			COPPER FROM HAMMOND'S TABLE	DIFFERENCE FROM HAMMOND'S TABLE (CALCULATED VALUES)		COPPER BY DICHROMATE (COLORIMETRIC)			
	FOUND	CALCULATED <sup>a</sup>	FOUND MINUS CALCU- LATED		mg.	per cent	FOUND	CALCULATED <sup>b</sup>	FOUND MINUS CALCULATED	DICHROMATE MINUS THIOCYANATE (CALCULATED VALUES)
mg.										
23	43.3	43.0	+0.3	43.4	-0.4	1.0	43.7	43.0	+0.7	0
46	85.5	85.3	+0.2	85.8	-0.5	0.6	85.2	85.4	-0.2	+0.1
69	126.9	127.0	-0.1	127.4	-0.4	0.3	127.4	127.1	+0.3	+0.1
92	168.1	168.1	0	168.4	-0.3	0.2	168.2	168.1	+0.1	0
115	208.5	208.5	0	208.7	-0.2	0.1	208.2	208.5	-0.3	0
138	248.2	248.3	-0.1	248.5	-0.2	0.1	248.3	248.3	0	0
161	287.6	287.5	+0.1	287.5	0	0	287.1	287.4	-0.3	-0.1
184	326.2	326.1	+0.1	326.0	+0.1	0	325.9	325.9	0	-0.2
207	363.9	364.0	-0.1	363.8	+0.2	0.1	363.6	363.7	-0.1	-0.3
230	401.5	401.3	+0.2	400.9	+0.4	0.1	401.3	400.9	+0.4	-0.4
243	421.9	422.1	-0.2	421.1	+1.0	0.2	421.4	421.6	-0.2	-0.5

<sup>a</sup> Formula (5) Cu (by thiocyanate) = 1.8318 | -0.000596 |<sup>2</sup><sup>b</sup> Formula (6) Cu (by dichromate) = 1.8940 | -0.000614 |<sup>2</sup>.

TABLE 8.—*Milligrams of copper reduced by invert sugar*

WEIGHT OF INVERT SUGAR	COPPER BY THIOSULFATE			COPPER FROM HAMMOND'S TABLE	DIFFERENCE FROM HAMMOND'S TABLE (CALCULATED VALUES)		COPPER BY DICHROMATE			
	FOUND	CALCULATED <sup>a</sup>	FOUND MINUS CALCULATED		mg.	per cent	FOUND	CALCULATED <sup>b</sup>	FOUND MINUS CALCULATED	DICHROMATE MINUS THIOSULFATE (CALCULATED VALUES)
mg.										
23	45.0	45.2	-0.2	45.2	0	0	45.2	45.1	+0.1	-0.1
46	89.3	89.5	-0.2	89.6	-0.1	0.1	89.1	89.4	-0.3	-0.1
69	132.5	133.0	-0.5	133.0	0	0	132.3	132.7	-0.4	-0.3
92	175.1	175.5	-0.4	175.6	-0.1	0.1	175.5	175.3	+0.2	-0.2
115	217.3	217.3	0	217.2	+0.1	0	217.0	216.9	+0.1	-0.4
138	258.1	258.1	0	258.0	+0.1	0	257.3	257.7	-0.4	-0.4
161	298.4	298.1	+0.3	298.0	+0.1	0	298.1	297.5	-0.4	-0.6
184	337.2	337.2	0	337.0	+0.2	0.1	336.4	336.5	-0.1	-0.7
207	375.9	375.5	+0.4	375.2	+0.3	0.1	374.9	374.7	+0.2	-0.8
230	412.7	412.9	-0.2	412.4	+0.5	0.1	411.6	411.9	-0.3	-1.0

<sup>a</sup> Formula (7) Cu (by thiosulfate) = 1.98341 - 0.000818  $\frac{1}{2}$ .<sup>b</sup> Formula (8) Cu (by dichromate) = 1.98281 - 0.000827  $\frac{1}{2}$ .

duce less copper (22). This theory is completely in accord with the very exact results of Quisumbing and Thomas (23), whose deviations from the rule of mixtures are in the same direction and of about the same magnitude as those shown in this paper.

The weight of copper precipitated in all methods in which the reduction is carried out at the boiling point is influenced by the barometric pressure. During the experiments described here a complete record of the barometric readings was kept. When a considerable change in pressure occurred its effect was definitely noticeable in the amount of copper precipitated. In extreme cases the determination was repeated when more favorable atmospheric pressures obtained. The mean barometric reading for dextrose was 757 mm.; for levulose, 756 mm.; and for invert sugar, 752 mm. (corrected to 0° C. and for latitude).

The data in Tables 6-8 permit an appraisal of the dichromate method. Compared with the thiosulfate method the results are slightly low. In the dextrose table there is an average deficiency of 0.14 per cent; in the invert sugar table of 0.19 per cent; and in the levulose table of 0.05 per cent. The mean deficiency in all 31 analyses is 0.13 per cent. If this difference should prove to be constant it could readily be corrected empirically, most simply by diminishing the concentration of dichromate in the standard solution by 0.13 per cent. The residuals would then have both positive and negative signs, and the errors would be of the order of 0.1 per cent.

#### IV. COMPARISON OF DATA WITH THOSE OF HAMMOND

In Tables 6, 7, and 8 are shown the deviations of the results from those of Hammond, the difference being given both in milligrams and in percentage of the copper reduced. A definite trend is noticeable, the results of the present measurements being in general slightly lower than Hammond's at the low concentrations of sugar and higher at the high concentrations. This suggests some small systematic difference in procedure. In conference it was revealed that Hammond's crucibles were probably more closely packed than were those used by the writers, thus causing a slight increase in the time required for filtration. Hammond required 90-100 seconds to filter the reaction mixture, while the writers required 30-50 seconds.

At the end of the 2-minute period of boiling there are two competing reactions that occur during the filtration. One is the back oxidation of cuprous oxide by air, resulting in re-solution of precipitated copper; the other is the continued reduction of copper. The additional amount of copper reduced by an extension of time of the reaction is dependent on the amount of sugar taken for analysis. Thus, as shown in Table 9, the weight of reduced copper increases considerably with extension of time for low concentrations of dextrose, less so for intermediate concentrations, and not at all for high concentrations.

The slower filtration in Hammond's analyses is equivalent to a slight

extension of reaction time. Hence at low concentrations of sugar he obtains, as expected, slightly greater reduction of copper. Throughout the wide range of intermediate concentrations these two competing effects counterbalance each other and the agreement between the two sets of analyses is highly satisfactory. In the range of the greater weights of sugar the only remaining effect of delayed filtration is the back oxidation by air, and thus Hammond obtains slightly less copper than do the writers.

TABLE 9.—*Effect of duration of boiling on the amount of copper precipitated at varying concentrations of sugar*

DEXTROSE (MG.)	46	138	230
DURATION OF BOILING	COPPER		
minutes	mg.	mg.	mg.
1	91.4	265.3	424.3
2	93.5	268.1	423.9
3	94.8	269.0	424.6
4.5	96.9	271.6	423.9
6	98.5	273.7	424.6

It would perhaps be possible to add more closely defined specifications for the Munson and Walker method. This appears inadvisable, however, since it would detract from the simplicity of the method.

A comparison of these two investigations serves fairly to evaluate the precision obtainable by Munson and Walker's method since they represent two quite independent series of analyses. The percentage deviations of the writers' values from those of Hammond are given in the respective columns of Tables 6-8. Of the 31 values given, 19 (61 per cent) are in agreement within 0.1 per cent, and 25 (81 per cent) within 0.2 per cent. The remaining 6 values are all at the extreme high or low concentrations of sugar. All the values for invert sugar and 6 of the 11 values for levulose agree within 0.1 per cent. Curiously enough the deviations are greater in the dextrose analyses than in those of the other sugars. The mean difference from Hammond throughout the whole series of three sugars is, regardless of sign, 0.19 per cent. The greater part of this difference is contributed by the extreme high and low concentrations of sugar.

Munson and Walker's procedure is essentially a macro-method. If, therefore, attention is confined to those determinations where there is sufficient precipitation to insure accuracy, and not so great a precipitation that the copper approaches exhaustion, say between 69 and 207 mg. of sugar, there will be found an average deviation from Hammond of 0.5 mg. (0.17 per cent) for dextrose, 0.2 mg. (0.11 per cent) for levulose, and 0.1 mg. (0.05 per cent) for invert sugar. The mean deviation for all three sugars within this range of concentrations is 0.26 mg. (0.11 per cent).

It is probable that a close collaboration would result in a reconciliation



of the small differences observed, but the value of such an attempt would be problematical. The conclusion seems justified that the copper equivalents found in the present investigation corroborate those of Hammond well within the limits of experimental error. It is recommended, therefore, that Hammond's tables be substituted for those of Munson and Walker in all analyses where the reduced copper is determined analytically.

Of the sucrose-invert sugar mixtures, only a small number of analyses were undertaken for the purposes of verifying Hammond's data and assisting in making a decision between his data and those of Erb and Zerbán (2). These latter authors have established formulas relating copper to 0.4 gram of mixtures of sucrose and invert sugar. At the higher concentrations of invert sugar they are in almost perfect agreement with Hammond, but at certain lower concentrations their values diverge by a maximum of 1.7 mg. of copper. Since their discrepant values are in agreement with Munson and Walker's copper precipitates, which as has been shown above, are contaminated, they are probably too high.

In Table 10 are given the weights of copper obtained from various sucrose-invert sugar mixtures. These values follow the general tendency found in the previous analyses, an agreement with Hammond at the middle range of concentrations and a slightly greater recovery of copper at the high concentrations. In the analysis of 0.4 gram of total sugar the results obtained by the writers are in practically perfect agreement with those of Hammond.

TABLE 10.—*Copper equivalents of some sucrose-invert sugar mixtures*

TOTAL SUGAR	SUCROSE	INVERT SUGAR	BY THIOSULFATE	BY ELECTROLYSIS	FROM HAMMOND'S TABLE	FROM ERB AND ZERBAN'S FORMULA	BY DICHROMATE	
							COLORIMETRIC	ELECTROMETRIC
grams	grams	mg.	mg.	mg.	mg.	mg.	mg.	mg.
2.0	1.880	120	—	238.9	238.7	—	238.8	238.6
2.0	1.793	207	385.6	384.7	384.2	—	—	—
2.0	1.770	230	421.6	421.2	420.3	—	—	—
0.4	0.340	60	156.4	—	156.5	158.2	156.9	156.8
0.4	0.320	80	119.6	—	119.7	121.3	—	—

## V. SUMMARY AND CONCLUSIONS

In a recent revision of the Munson and Walker reducing-sugar tables (1) by Hammond (3) important deviations in many of the copper values were disclosed. The present investigation, constituting a third series of analyses, corroborates Hammond's measurements within the limits of experimental error. The differences between Hammond's and Munson and Walker's values for copper are due almost entirely to the respective methods of estimating copper. Hammond determined reduced copper by electrolysis, while Munson and Walker weighed the precipitated cuprous

oxide. It is now shown that cuprous oxide is contaminated with organic decomposition products even when pure sugars are analyzed. The amount of contamination is found to be almost exactly equal to the difference between Hammond's and Munson and Walker's copper values.

The writers have shown that reduced copper should be determined by analysis and not by direct weighing of cuprous oxide and that Hammond's tables should be substituted for those of Munson and Walker.

Erb and Zerban's analyses of 0.4 gram of sucrose-invert sugar mixtures are in agreement with those of Hammond except within a short range of lower concentrations. The analyses here presented are in agreement with Hammond's within this range.

The various methods for the determination of copper are discussed. The main reliance during the investigation was the iodometric method in acetic acid solution with the concentration (4.2 grams per 100 ml.) of potassium iodide specified by Shaffer and Hartmann (10) and with the addition of thiocyanate at the end of the titration as specified by Foote and Vance (11).

A method of determining cuprous oxide by oxidation with an excess of dichromate and back titration with ferrous sulfate to a colorimetric or electrometric end point is described.

The permanganate method as modified by Schoorl and Regenbogen (15) is shown to give accurate results.

The precision of Munson and Walker's method as indicated by Hammond's and the writers' independent analyses is shown to be about 0.2 per cent. If the concentrations of sugar are restricted to the range between 69 and 207 mg. of reducing sugar, the average precision appears to be about 0.1 per cent.

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## STUDIES OF FLORIDA AND CALIFORNIA ORANGES IN REGARD TO THE RELATIONSHIP OF FROST DAMAGE TO JUICE CONTENT\*

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Oranges that have been injured on the trees by freezing are characterized by drying out of the flesh, which continues progressively for a considerable period, in some cases several months, after the freezing occurs. The drying may occur at any place in the orange, although it generally begins at the stem and progresses towards the center portion. This is especially true of Florida fruit. In California fruit drying often occurs at the blossom end or at the center without being present at the stem end. Any drying is accompanied by a decidedly lowered juice content.

In the sections where oranges are grown it has been the custom for many years to make an approximate determination of the extent of injury due to freezing by estimating the area of dried tissue in the surface exposed by one or more transverse cuts. These cuts have in all cases been made at right angles to the axis that passes from stem to blossom end, but their location along this axis has varied from time to time and from place to place, being in some instances one-quarter to one-half inch below the surface of the flesh at the stem end, half way between stem or blossom end and center, and at the center. This method of determining the extent of injury has not been entirely satisfactory for several reasons. The dried portion of an orange is frequently irregular in shape and the area exposed by a transverse cut does not accurately indicate the volume occupied by the dried material. The dried area usually shades off gradually into the undried portion of the fruit, making estimation difficult, with the result that different people examining the same fruit may make varying estimations of the extent of drying.

Since there are present in any lot of oranges that have been commercially

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packed a number of sound, uninjured oranges, it was thought that a comparison between the amount of juice obtained from the sound oranges and the amount obtained from those that showed evidence of having suffered injury would indicate the extent of such injury. This idea is based on the proposition that the sound oranges contain their normal amount of juice, and that if no injury from freezing had occurred the remainder of the fruit in the lot would have had the same proportion of juice. The extent to which the juice content of the injured fruit falls below this normal juice content, as indicated by the sound fruit, is the extent to which the fruit has in fact been injured.

Accordingly, investigational work was done in Florida in 1940 and in California in 1941 to determine the value of this method. The work in Florida was done on several varieties of midseason fruit, and on Valencias. Approximately 4500 Florida oranges were examined. Samples were obtained from packing houses located in widely scattered parts of the Florida area that was affected by the freeze of that year. These extended from Citra in the North to Ft. Pierce in the South, and from Cocoa on the East Coast to Lake Wales in the Ridge Section. The samples, usually  $\frac{3}{8}$  of a bushel, were taken at random from the packing-house stock and at different points in the sorting and packing process. Each sample was cut until 25 sound and 25 injured fruit were obtained. If 25 oranges of either kind were not available, all that were present in the sample and 25 fruit of the other kind were examined. Each orange was cut at a point approximately  $\frac{1}{4}$  inch below the surface of the flesh at the stem end, and again at the center. If any evidence of drying was visible the orange was classed as injured, and the dry area was estimated in terms of the per cent of the total exposed surface that the dried area occupied. It was considered inadvisable to attempt to make such estimations closer than within a range of 10 per cent and so the estimates of drying were recorded as 0 to 10, 10 to 20, 20 to 30, and over 30 per cent, as the case might be. Each orange was weighed, and the juice was extracted by reaming, strained through a wire strainer, and weighed. The proportion (by weight) of juice in the total sample, and the proportion in the sound fruit only, were then calculated. When the juice content of the total sample was compared with the normal juice content, that is, the juice content of the sound fruit only, and the comparison was expressed on a percentage basis, it was found, as would be expected, that the juice content of the sample fell progressively below the normal juice content as the extent of drying observed in the fruit increased.

In order to ascertain whether oranges that had been exposed to freezing temperatures still retained their normal juice content when there was no visible evidence of injury, the average of the juice content of the sound oranges in the samples examined in 1940 was compared with the average of the juice content of samples of 25 oranges of the same variety, grown in the same locations in 1936, a year when no freezing weather occurred. The

results (Table 1) indicate that the range of juice content of each of the different varieties in both years was substantially the same.

TABLE 1.—*Juice content of Florida oranges (per cent by weight)*

VARIETY	1939-40			1936-37		
	NO. SAMPLES	LOW	HIGH	NO. SAMPLES	LOW	HIGH
Pineapples	20	45.5	54.2	14	47.8	55.6
Seedlings	12	47.0	53.9	16	46.2	53.9
Valencias	30	48.0	59.7	27	51.9	59.1

In 1941, frozen Valencias were available for study in the Tulare section of California, and examination was made of approximately 5000 oranges. The work done there differed only slightly from that done in Florida. Only Valencias were available, and the area from which samples were drawn was not so extensive as that in Florida. The samples consisted of 100 fruit, all of which were examined, but no attempt was made to adjust the number of sound or injured fruit. Since drying sometimes occurs in the blossom half of California fruit, a third cut corresponding to the stem end cut, was made in the blossom end. Estimates of the dried area were made as in the Florida work. Determinations of juice content were not made on individual fruit, but only on the groups of sound and injured oranges occurring in each sample. The juice was extracted by reaming as in Florida, but was strained and pressed "dry" through cheese cloth instead of being passed through a wire strainer. A basket-type centrifuge was used to free the juice from seeds and tissue in some of the samples, but straining through cheese cloth seemed to give equally good results and had the advantage of being more rapid.

For the purpose of determining whether or not the presence of fruit of mixed size within a sample would cause any difficulty, a quantity of completely sound fruit of different sizes was obtained from a single source. This fruit was grouped in various ways and examined. The results obtained with these samples, as well as with many other samples of mixed sizes in which injured fruit was present, indicate that the method gives as satisfactory results on fruit that is not of uniform size as it does on fruit that has been graded for size.

The results obtained in California, definitely in line with those obtained in Florida, indicate that the greater the amount of drying observed in the fruit the lower is the juice content of the total sample in comparison with the normal juice content as indicated by the sound fruit in the sample. It is difficult visually to evaluate and compare the relative extent of drying in several samples, some of which may have a few badly dried fruit, while others have many that are only slightly dried, but an attempt has been made to estimate the average amount of drying observed in each sample by arbitrarily taking the midpoints in the estimation of damage at each

of the three cuts made on the individual orange, and averaging them. For example, an orange in which the drying at the three cuts was estimated as 0-10, 10-20, 10-20 would be considered to have an average amount of drying of  $(5+15+15) \div 3 = 12$  per cent. If the dried area was estimated as greater than 30 per cent on any cut surface, the figure 50 per cent was arbitrarily taken. The averages of the individual oranges were added and

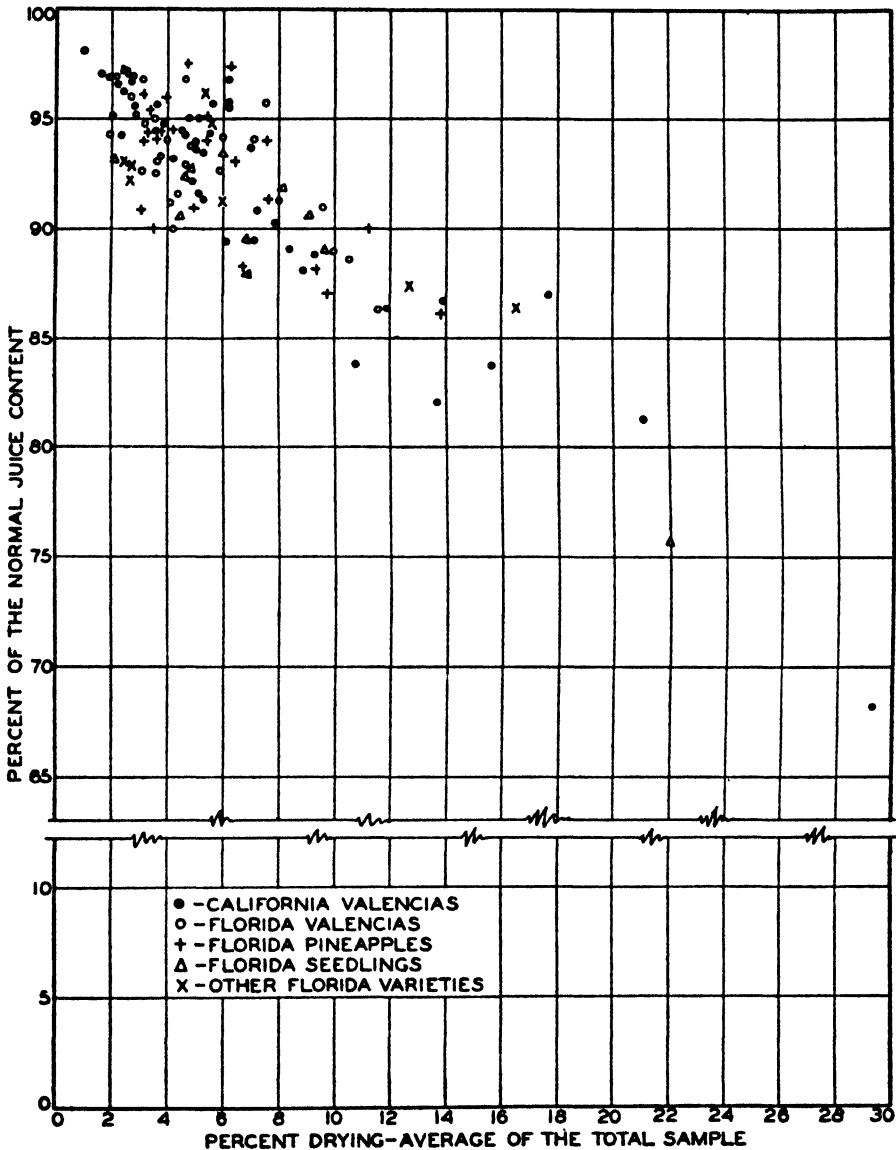


CHART 1

divided by the total number of oranges in the sample to obtain the average drying in the entire sample. In the case of Florida oranges, where only two cuts were made, the amount of drying observed at these cuts was also divided by 3 to obtain a comparable estimate of the average amount of drying in the orange, since it could be reasoned that if a third cut had been made at the blossom end, no drying would have been observed, and hence the total amount would not have been increased.

In Chart 1 the averages of the estimates of drying are plotted against the percentages of the normal juice obtained from the sample. It will be noted that the Florida and California fruit, which includes different varieties, different root stocks and different commercial sizes, forms the same pattern, and that a well defined trend shows fairly close agreement between the average estimated drying in the sample and the loss in juice that accompanies this drying.

Table 2, based on Florida fruit, shows a comparison between the dried area at the stem end and at center cuts and the corresponding juice content, expressed as percentage of the normal juice content. The relationship between the extent of dried area and the accompanying lowering in juice content is quite marked.

As a result of the studies conducted on Florida and California oranges it appears that a comparison between the juice content of the sound fruit in a given lot of oranges and the juice content of all the fruit present in the lot may be used for determining objectively the extent to which the lot has been injured by freezing.

TABLE 2.—Comparison between observed extent of drying and juice yield in terms of per cent of normal juice content.\* Florida oranges—1940

DRYING AT STEM END	DRYING AT CENTER CUT (PER CENT)				
	0	0-10	10-20	20-30	30+
<i>per cent</i>					
<i>Pineapple Oranges</i>					
		(1)			
0	100	79.2			
	(93)	(11)			
0-10	97.9	90.6			
	(82)	(24)		(1)	
10-20	96.9	92.7		71.9	
	(60)	(32)	(1)		
20-30	95.8	89.6	68.8		
	(76)	(78)	(9)	(1)	(3)
30+	94.8	89.6	83.3	79.2	78.1
<i>Indian River</i>					
0	100				
	(27)	(4)			
0-10	98.0	94.1			
	(10)	(1)			
10-20	95.1	96.1			

TABLE 2.—Continued

DRYING AT STEM END	DRYING AT CENTER CUT (PER CENT)				
	0	0-10	20-30	20-30	30+
<i>per cent</i>					
	(1)	(3)			
20-30	94.1	92.2			
	(2)	(2)			
30+	91.2	93.1			
<i>Seedlings</i>					
		(1)			
0	100	96.0			
	(34)	(3)			
0-10	96.0	90.0			
	(43)	(9)	(1)		
10-20	94.0	93.0	88.0		
	(42)	(18)	(1)		
20-30	94.0	90.0	95.0		
	(58)	(63)	(11)		
30+	91.0	88.0	86.0	(8)	(7)
				83.0	71.0
<i>Maltese Seedless</i>					
0	100				
	(35)	(20)			
0-10	95.2	93.3			
	(26)	(9)			
10-20	94.2	90.4			
	(25)	(6)	(1)		
20-30	94.2	91.3	87.5		
	(32)	(1)	(2)	(2)	
30+	91.3	89.4	80.8	73.1	
<i>Valencias</i>					
		(1)	(1)		
0	100	97.2	95.4		
	(183)	(13)			
0-10	96.3	91.7			
	(110)	(11)			
10-20	94.4	93.5			
	(113)	(13)	(2)		
20-30	95.4	90.7	87.0		
	(165)	(39)	(5)		
30+	92.6	88.9	78.7		
<i>All Oranges</i>					
		(3)	(1)		
0	100	91.2	98.0		
	(372)	(51)			
0-10	97.1	93.1			
	(271)	(54)	(1)	(1)	
10-20	95.1	92.2	87.3	70.6	
	(241)	(72)	(5)		
20-30	95.1	89.2	86.3		
	(333)	(199)	(27)	(11)	(10)
30+	93.1	88.2	83.3	80.4	72.5

\* Figures in parentheses indicate number of oranges.



## FOOD COLOR SOLUTIONS AS POSSIBLE CONTAMINANTS OF FOOD PRODUCTS

By WILLIAM A. BOYLES (Division of Bacteriology, U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The idea that food colors are heavily contaminated with bacteria is not prevalent. Investigations of the relation of the constituents of ice cream to the sanitary quality of the finished product have demonstrated, however, that color solutions often carry excessive loads of bacteria. Tracy and Brown (7) reported that 12 per cent of the samples of color solutions taken from 21 different ice cream plants contained more than 1,000,000 bacteria per ml. and that 12.6 per cent contained *Escherichia coli*. Newman and Reynolds (2) found counts as high as 16,000,000 organisms per ml. Fabian (1) reported counts as high as 15,000,000 and also found that 35 per cent of the samples examined contained bacteria of the *Escherichia-Aerobacter* group. Smallfield (5) reported counts as high as 198,000,000 and suggested that dye solutions might increase the load in ice cream by 100,000 per gram. Prucha (3) found only limited numbers of bacteria in dye powders but much greater numbers in dye solutions.

Numbers of organisms of these magnitudes emphasize the important role dye solutions might play in the contamination of food products.

In the study made by the writer unopened samples of color solutions collected from stores and wholesale houses were examined, as well as samples submitted to the color laboratory of the Food and Drug Administration for certification. A total of 98 samples of color solutions from 13 manufacturers or distributors was examined for members of the coliform group, thermophiles, and anaerobes, and the total bacterial counts were determined.

## PROCEDURE

For the isolation of coliform bacteria, the colors were seeded in serial dilutions into lactose broth prepared according to Standard Methods of Water Analysis (1933) (6), and cultures showing gas in 24 or 48 hours were streaked onto eosin methylene blue agar. The colors were also inoculated into meat medium for anaerobes and into bromocresol purple dextrose broth for thermophiles of the flat sour type. Bacterial counts were obtained on plain agar, and all colonies were purified by replating two or three times before further studies were made. The exterior of all samples was treated with 75 per cent alcohol before the samples were opened to remove subsamples.

## RESULTS

The results are summarized in Table 1. Thermophiles and clostridia were not found.

TABLE 1.—*Bacterial counts and incidence of coliform organisms in food colors*

DISTRIBUTORS	NUMBER OF SAMPLES	NUMBER OF COLORS	MAXIMUM COUNT PER ML.	SAMPLES CONTAINING COLIFORMS		SAMPLES WITH COUNTS OF 50,000 OR OVER		SAMPLES WITH COUNT OF 400,000 OR OVER	
				No.	Per cent	No.	No. containing coliforms	No.	No. containing coliforms
1	23	4	178,000			6			
2	5	3	19,000						
3	20	5	8,700,000	7	35	13	6	11	5
4	17	16	12,100,000	5	29.4	12	5	12	5
5	5	5	26,000,000	3	60	2	1	1	1
6	3	3	Under 10						
7	5	5	110	1	20				
8	7	7	Under 10						
9	1	1	450,000			1		1	
10	1	1	Under 10						
11	4	4	1,660,000	1	25	1	1	1	1
12	3	3	Under 10						
13	4	4	32,000						

The number of microorganisms in all samples of liquid dyes was found to range from less than 10 to 26,000,000 per ml. Twenty-six of the 98 samples (26.5 per cent) contained over 400,000 organisms per ml. Seventeen (17.3 per cent) contained members of the coliform group, of which two were a slow lactose-fermenting variety of *Escherichia coli.*, present in one sample only.

Bacterial counts of 26 samples, which had been in wholesale houses an indefinite length of time and from two distributors, ranged from less than 10 to 178,000 per ml. No bacteria of the coliform group were found in these samples. Sixty-three samples from fresh stock had a bacterial content ranging from less than 10 to 26,000,000 per ml., and 15 of these samples contained bacteria of the coliform group.

High bacterial counts were found in some dyes that were a year or more old. One of nine samples collected at one wholesale house, where they had stood on a shelf at least a year, showed a count as high as 4,430,000. Others contained bacteria in excess of 60,000 per ml., and organisms of the coliform group were found in two of these nine samples.

The fresh stock of dye solutions examined that showed high bacterial counts was divided among samples from four distributors, and these samples had a greater incidence of members of the coliform group than did any of the so-called old stock examined. Thirteen of 20 samples from one distributor had counts in excess of 50,000, and seven of these 20 samples contained members of the coliform group; 12 of 17 samples from the second distributor had counts in excess of 400,000, and five of these 17 contained members of the coliform group; 2 of 5 samples from a third distributor contained more than 50,000 bacteria per ml. and 3 of these 5 samples

contained coliform bacteria; and 1 of 4 samples from the fourth distributor had a count of 1,660,000 and contained coliform bacteria.

All colors or shades of color were found to contain numbers of micro-organisms. That color or shade of color bears no relationship to the bacterial load is illustrated by the data in Table 2.

TABLE 2.—*Distribution of high bacterial counts among colors*

	COLOR OR SHADE	NO. OF SAMPLES WITH COUNT EXCEEDING 50,000	NO. CONTAINING COLIFORM BACTERIA	RANGE OF COUNTS
Distributor No. 3	Grape	5	3	60,000 to 7,800,000
	Green	3	0	30,000 to 940,000
	Yellow	1	0	3,230,000
	Red	2	1	1,150,000 to 2,120,000
	Orange	2	2	570,000 to 3,850,000
Distributor No. 4	Yellow	3	2	400,000 to 8,000,000
	Blue	1	0	1,540,000
	Violet	1	0	12,100,000
	Orange	2	0	460,000 to 5,600,000
	Brown	1	1	8,300,000
	Rose	2	2	2,800,000 to 8,100,000
	Raspberry	1	0	1,500,000
	Tomato	1	0	4,000,000
Distributor No. 5	Red	1	1	26,000,000
	Green	1	0	160,000

To determine the predominating types of bacteria present in liquid dyes representative colonies were chosen from 43 samples of liquid dyes from eight distributors; 235 organisms were examined. Twenty-five were yeasts that predominated in four dye solutions. The following types of bacteria were found: *Achromobacter*, 117; *Aerobacter*, 45; *Pseudomonas*, 15; *Citrobacter*, 10; *Flavobacterium*, 5; *Bacillus*, 5; *Micrococcus*, 5; *Serratia*, 3; *Escherichia*, 2; *Rhodococcus*, 1; Genus unidentified, 2. The slow lactose-fermenting varieties of all three types (*Escherichia*, *Citrobacter*, *Aerobacter*) of the coliform group were encountered.

To obtain some idea of the possible inhibitory action of dye solutions, 1 per cent solutions of 13 water-soluble dyes were prepared and tested against suspensions of *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* by the agar cup method of Ruehle and Brewer (4). Only a few of the

dyes exhibited definite selective or inhibitory action. Erythrosine gave definite zones of inhibitory action with *Bacillus cereus* and *Staphylococcus aureus* but not with *Escherichia coli*. Orange I gave definite zones of inhibitory action with *Escherichia coli* and *Staphylococcus aureus*, but was only slightly inhibitory to *Bacillus cereus*.

Indefinite results were obtained with other dyes. Indigo was reduced by *Bacillus cereus* and slightly reduced by *Escherichia coli* and *Staphylococcus aureus*.

### DISCUSSION

Some degree of correlation exists in dye solutions between high counts of bacteria and the presence of coliform organisms. An examination of the data in Table 1 shows that of the samples in which coliform bacteria were found, 13 contained counts in excess of 50,000 organisms per ml., and 12 samples in which coliform bacteria were found contained 400,000 organisms or more per ml. Of 17 samples containing coliform bacteria, 16 were obtained from four distributors.

Prucha (3) found only limited numbers of bacteria in dye powders, and the small number of dry dyes examined in this investigation confirms his findings. However, dye solutions have repeatedly been shown to contain excessive numbers of bacteria. In this study such excessive numbers are surprising because all the samples of liquid colors were received in closed containers that gave adequate protection against bacterial contamination. Consequently, excessive bacterial contamination could result only from the manner in which the product was treated in the plant.

Some of the bacterial counts made during this investigation, as well as those reported by other investigators, are as large as those found at times in foods that are conducive to bacterial growth, and regardless of whether or not they are accompanied by coliform bacteria, there is a very apparent significance attached to such findings. A count in excess of 50,000 is high for a product in which the available nutriment for bacteria is limited and which receives in the manufacturing process a treatment that is extremely detrimental to microbial life. Bacterial counts in excess of 400,000, which were made in samples from 5 of the 13 manufacturers whose products were examined, must be considered to be evidence of negligence and dirty plant practices. Although pasteurization would eliminate most of the bacteria in dye products, it cannot be considered a substitute for good plant practice, nor does it remove the insanitary conditions under which such heavily contaminated products are manufactured.

### SUMMARY AND CONCLUSIONS

(1) Ninety-eight samples of dyes were examined; the total content of microorganisms ranged from less than 10 per ml. to 26,000,000.

(2) Twenty-six of 98 samples (26.5 per cent) contained over 400,000

organisms per ml. Seventeen (17.3 per cent) contained members of the coliform group.

(3) Clostridia, molds, and thermophiles were absent from all samples.

(4) High bacterial counts were found in dyes known to be one year or more old and were not restricted to any colors or shades of color.

(5) Some correlation was noted between the excessive numbers of bacteria found in samples from four distributors and the presence of coliform bacteria.

(6) Of 13 water-soluble food colors, only erythrosine and orange I showed definite inhibitory properties.

(7) The combination of high bacterial content and coliform bacteria indicates that some dye solutions are prepared under insanitary conditions.

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## NOTES

### Note on Experiences with the Hendrey Method for Determination of Soybean Flour in Sausages\*

The Massachusetts law relative to the sale of sausages and sausage meat provides that such material shall be considered to be adulterated if it contains any cereal or vegetable flour or any product thereof in excess of 2 per cent. An exception is made of a purely vegetable sausage sold as such and containing not less than 20 per cent of vegetables or vegetable products.

Because of the absence of a satisfactory method for the determination of soybean flour in meat products, sausages containing soybean flour to an amount considerably in excess of the 2 per cent permitted by law have been manufactured and sold in Massachusetts. A method devised to detect the presence and quantity of this material is needed.

Hendrey proposes a method<sup>1</sup> based on the fact that commercial soybean flours contain approximately 10 per cent of insoluble nonfermentable sugars. Sugars of this character are found to a very limited extent in meats, e.g., beef and pork, and in much larger quantities in certain materials high in pentosans, arabans, etc., such as whole wheat flour; but they are not found in vegetable products commonly used in the manufacture of sausages.

The method involves elimination of the moisture and fat in the sample, thorough washing with 50 per cent alcohol for the complete removal of soluble sugars, subsequent hydrolysis of the insoluble sugars, and fermentation of the hydrolyzed material with yeast. The copper-reducing substances remaining after the fermentation are determined as invert sugar, from which soybean flour is calculated by the factor 9.4. Check results can be secured on separate portions of the well-mixed sample.

The method was tested on samples of known composition from three plants where the course of manufacture and the ingredients were observed through to the finished product. The results on such authentic samples follow.

Plant A used beef, pork, salt, ice, spices, and skimmed-milk powder, but no soybean or other vegetable flour. Analysis of the finished product showed insoluble nonfermentable sugars equivalent to 1.7 per cent of soybean flour.

Plant B used soybean flour to the extent of 1.24 per cent by weight in the finished product, together with beef, pork, spices, ice, skimmed-milk powder, cane sugar, and cure. Analysis of the finished product gave in-

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<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 611 (1939).

soluble nonfermentable sugars equivalent to 2.34 per cent of soybean flour. In this instance the calculated blank was 1.10 per cent.

Plant C did not use any soybean flour in this particular batch. The product contained insoluble nonfermentable sugars that calculated to 1.05 per cent soybean flour.

The weakest point in the method is the fact that it does not actually identify soybean flour. It merely determines the insoluble nonfermentable sugars such as are present in soybean flour. The length of time required for the analysis and the difficulty of completely washing out the soluble sugars, particularly lactose, which may have been introduced by the addition of skimmed-milk powder, constitute other disadvantages. It is necessary that the sample be ground very fine in a mortar prior to washing with 50 per cent alcohol, particularly when skimmed-milk powder is present. It is also necessary to use a Büchner funnel and employ suction during the filtration. The addition of Filter Cel to the paper prior to filtration is a decided aid when suction is employed. It was found that the washing outlined in the method did not remove all the soluble sugars, but that washing to a total volume of at least 400–500 ml. was necessary. The last washings were evaporated to a small volume and tested with Benedict's solution for the presence of reducing sugars. A negative test was secured only after repeated washings.

Another possible source of error is the fact that the spices used in the manufacture of smoked sausages, such as frankforts and bologna, contain certain insoluble nonfermentable sugars. One such sample of spice was found to contain nonfermentable sugars that calculated to 8.8 per cent soybean flour. Three samples of starch-containing binders obtained from sausage plants and a sample of skimmed-milk powder gave negative results by the Hendrey method for the presence of soybean flour.

As a result of these investigations, the writers concluded that the Hendrey method, when carefully followed and when proper allowance had been made for the presence of insoluble nonfermentable sugars from other sources, is sufficiently accurate for law-enforcement purposes, but that a shorter, more specific method would be desirable. Since the method will not identify soybean flour, confirmatory evidence is necessary, and means to this end would seem to be the biological precipitin test. With evidence that the sample actually contains soybean flour, the amount of such flour can be determined by the Hendrey method.

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**SECOND DAY**  
**TUESDAY—MORNING AND AFTERNOON**  
**SESSIONS—*Continued***

**REPORT ON SYNTHETIC DRUGS**

By L. E. WARREN (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

Last year, as the Chairman has stated, the Section on Drugs was divided into four subsections, each under a different referee. Reports will now be given on one of these subdivisions, that of Synthetic Drugs.

Six topics were assigned for study; namely benzedrine; hydroxyquinoline sulfate; methylene blue; aminopyrine, acetophenetidin, and caffeine; ethyl aminobenzoate; and sulfapyridine.

Of these, the associate referees for two, hydroxyquinoline sulfate and ethyl aminobenzoate, reported that no work was done. The Referee recommends that these subjects be continued. Of the other four, the Referee recommends that one, aminopyrine, acetophenetidin, and caffeine, be closed with the adoption of the method of separation as tentative. The Referee recommends that the remaining three topics be continued.

During the past year, the Fifth Edition of *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists* has been published. The Fourth Edition included 90 topics under drugs, whereas the Fifth Edition has 105; but since three topics were deleted from the Fourth Edition the net increase is but 10. Among the newer subjects are: Acetylsalicylic acid, acetophenetidin and caffeine, cod liver oil in emulsions, identification of gums, hypophosphites in sirups, mandelic acid, nicotinic acid, sulfanilamide, and terpin hydrate and codeine in elixirs. However, the 13 new topics do not give a fair picture of all the new methods added. For example, in the Fourth Edition under a single topic, microchemical tests for alkaloids, tests for 26 alkaloids are described. In the Fifth Edition, the number of alkaloids for which tests are given is 39. Likewise, the Fourth Edition describes microchemical tests for but 12 synthetics, whereas the Fifth Edition has 20.

*Benzedrine* (desoxy-nor-ephedrine).—This topic was assigned two years ago, but no work was done last year owing to difficulties in securing authentic material. This year the associate referee considered three qualitative tests and three methods for the assay of the substance. One of the quantitative methods (a benzylation process) was selected for trial. The results were favorable, but no collaborative work was carried out. The associate referee recommends that the method be subjected to collaborative study during the coming year. The Referee concurs.

*Hydroxyquinoline sulfate*.—This topic has been under consideration



for several years, but no reports have been made. The Referee recommends that the topic be reassigned.

*Methylene blue*.—In 1923, the Association adopted a method for the assay of methylene blue that depends on titration with an excess of standardized iodine solution in a medium containing acetic acid. Several years ago there appeared in the literature a method that depends on the precipitation of the dye with a soluble perchlorate. At that time, an associate referee was appointed to investigate the new method. He recommended the retention of the A.O.A.C. procedure, *This Journal*, 22, 703 (1939). Since that time, the perchlorate method has been adopted by the U.S.P. The associate referee has continued his investigation of the two methods. Since the U.S.P. method does not provide for the separation of methylene blue from mixtures, the associate referee again recommends that the A.O.A.C. method be retained with the amendments given in his report.

The Referee concurs in these recommendations. Since the method is official, the changes should be adopted tentatively at this time. The Referee recommends further that the subject be discontinued.

*Acetophenetidin, aminopyrine, caffeine*.—Mixtures of two or more drugs are frequently used. The Association has adopted methods for the separation of several mixtures, such as acetanilid and caffeine; antipyrine and caffeine; acetylsalicylic acid and phenolphthalein; acetylsalicylic acid, acetophenetidin and caffeine; acetylsalicylic acid, acetophenetidin and salol; and others. At the last meeting, an associate referee was appointed to study the separation of acetophenetidin, aminopyrine, and caffeine. This year the associate referee and his collaborators devised a method based on the literature for the separation and determination of these drugs. The results obtained by six analysts are reasonably good. The associate referee recommends that the method be adopted as tentative. The Referee concurs. The Referee also recommends that mixtures of acetophenetidin, aminopyrine, caffeine, and phenobarbital be studied.

*Ethyl aminobenzoate (benzocaine)*.—This synthetic has been in use as a local anesthetic for many years. It has not previously been studied by the Association. An associate referee was appointed last year, but he reports that no work has been done. It is recommended that the subject be continued.

*Sulfapyridine*.—One of the newer synthetics that have come into considerable use in the last two or three years is sulfapyridine. It was studied this year by the Association for the first time. The associate referee adapted one of the methods in the literature (titration with standard sodium nitrite solution) to the assay of the pure substance and to a mixture. The results for the pure substance are good, but those for the mixture vary from 96.4 to 100 per cent of theory.

The associate referee recommends that further study be given to the subject. The Referee concurs.

*Ointment of mercuric nitrate.*—A method for the determination of mercury in this preparation was adopted two years ago, *This Journal*, 22, 96 (1939). In a contributed paper (see p. 927), R. K. Snyder states that he has an improvement on the A.O.A.C. tentative method that eliminates oxidation with permanganate. He dissolves the mercuric nitrate by heating with nitric acid as in the A.O.A.C. procedure and dilutes to volume. An aliquot portion of the solution is heated in a Kjeldahl flask to oxidize organic matter, and the cooled solution is titrated in the usual way. The advisability of appointing an associate referee to study this modification should be considered.

*Metrazol (cardiazol).*—Metrazol (pentamethylenetetrazol), one of the newer synthetics, has attained some prominence as a stimulant in place of camphor. Methods for its identification and determination are described in the literature, but none has been adopted by the Association. It is recommended that metrazol be studied.

*Sulfathiazole.*—One of the newer synthetics, sulfathiazole, is receiving favorable notice from the medical profession. It is related to sulfapyridine. The Referee recommends that sulfathiazole be studied.

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## REPORT ON BENZEDRINE

By JAMES H. CANNON (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

In accordance with the recommendation made last year, study of methods for the determination of benzedrine in inhalers was made. Limiting the study to methods for inhalers practically limits it to methods for the pure base, free from interfering substances, since the inhalers contain only minute quantities of oil of lavender and menthol in addition to the base (or the carbonate of the base). Therefore, in the work this year no consideration was given to possible schemes of separation from interfering substances. The following methods were considered by the Associate Referee:

### *Qualitative Tests*

1. Melting point of the benzoyl derivative, 134°–135° C. (NNR 1940).
2. Microchemical test with platonic chloride.
3. Coupling with *p*-nitrobenzenediazonium chloride. (*J. Pharmacol.*, 68, 419, (1940).

### *Quantitative Methods*

1. Distillation with steam, followed by titration (NNR 1940).
2. Colorimetric estimation with *p*-nitrobenzenediazonium chloride. (*J. Pharmacol.*, 68, 419 (1940).
3. Gravimetric determination by benzoylation.

Of the qualitative tests considered, the melting point determination on the benzoyl derivative seems preferable. This test is made on the

residue from the quantitative determination described later. The microchemical test with platonic chloride offers a quick identity check. This test is made by adding a drop of platonic chloride solution (A.O.A.C. microchemical reagent) to a drop of a 1-100 solution of benzedrine in dilute sulfuric acid on a microscope slide. Interlaced needles form quickly around the edge of the drop. A more sensitive test is the color test with *p*-nitrobenzenediazonium chloride. One disadvantage of this test lies in the fact that the reagent is not stable and must be freshly prepared.

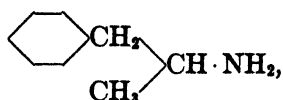
Available quantitative methods include titration of the base after distillation or extraction with ether, colorimetric determination after coupling with diazonium salts, and gravimetric determination of the benzoyl derivative. The last method was chosen for further study this year, for the following reasons:

1. It is a more direct method than the other two methods in that no special reagents or standard solutions are required.
2. The purity of the precipitate may be readily checked by melting point and nitrogen determinations.
3. Further qualitative testing is not required to establish the identity of the material being assayed.

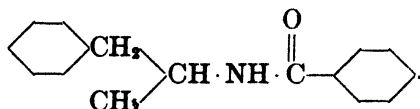
## EXPERIMENTAL

### OUTLINE OF GRAVIMETRIC METHOD

The gravimetric method depends upon the reaction between benzedrine, a substituted aliphatic amine,



and benzoyl chloride. The compound formed may be represented as follows:



The weight of the derivative therefore will be 1.770 times the weight of the benzedrine equivalent.

### PROCEDURE

(Applicable to pure solutions of salts of benzedrine.)

Place 30 ml. of 10% NaOH solution in a 125 ml. separator and add an aliquot of the benzedrine solution containing 50-100 mg. of benzedrine. Add 5 drops of benzoyl chloride and shake vigorously. Allow mixture to stand with occasional shaking for 10 minutes. Again add 5 drops of benzoyl chloride and shake occasionally for 10 minutes. Add a third 5-drop portion of benzoyl chloride, shake, and test the reaction of the solution with a piece of litmus. If necessary, add more NaOH solution to maintain alkalinity throughout the determination. Allow to stand 2 hours with occasional shaking. (At room temperature the complete hydrolysis of the benzoyl chloride seems to require considerable time.) Extract the solution with three 20 ml. portions of  $\text{CHCl}_3$ . Test for complete extraction, using a 10 ml. portion of

$\text{CHCl}_3$ . Combine the  $\text{CHCl}_3$  extracts in a second separator and wash with 5 ml. of water. Drain the  $\text{CHCl}_3$  through a pledget of cotton into a tared beaker. Carefully evaporate the  $\text{CHCl}_3$  just to dryness and allow the residue to attain a constant weight in the open air without further heating. Multiply the final weight of the residue by 0.565 to obtain the weight of benzedrine base in the aliquot.

#### DATA

(1) *Interferences*.—Ammonia, or amines that form chloroform-soluble compounds with benzoyl chloride will act as interfering substances. Benzamide, formed by the reaction with ammonia, is readily soluble in hot water and might be separated by a hot filtration of the precipitated benzoyl-benzedrine. Interference from esters such as methyl and ethyl benzoates, which might be formed during the benzoylation, may be avoided by a preliminary de-alcoholization in acid solution.

(2) *Range*.—The quantities of benzedrine specified in the method will be converted to the benzoyl derivative by the excess of benzoyl chloride called for. However, it has been found that incomplete benzoylation may be expected if much less benzoyl chloride is used, even though the amount is stoichiometrically much greater than is required for the quantity of benzedrine present. Possibly this is due to the fact that the hydrolysis of the reagent proceeds as rapidly as does the formation of the derivative. Smaller quantities of benzedrine than specified would of course be completely benzoylated and any excess benzoyl chloride would be completely hydrolyzed under the conditions of the test.

(3) *Accuracy and precision*.—Regarding the accuracy and precision of the proposed method, the following data are submitted:

*Sample*: A solution of pure benzedrine base (obtained from Smith, Klein, and French Laboratories, Philadelphia, Pa.) dissolved in dilute sulfuric acid.

The results follow:

SAMPLE	BENZEDRINE CALC'D FROM TOTAL N ON AN ALIQUOT OF SAMPLE SOLN	BENZEDRINE BY PROPOSED METHOD	RECOVERY	BENZEDRINE BY TOTAL NITROGEN ON THE BENZOYL DERIVATIVE OBTAINED	RECOVERY
	mg./ml.	mg./ml.	per cent	mg./ml.	per cent
A	9.61	9.86	103	9.50	99
	9.61	9.82	102	9.70	101
	9.61	9.50	99	8.84*	92
		9.73 av.	101 av.	9.35 av.	97.3 av.
B	10.8	11.3	105	10.8	100
	10.8	10.8	100	†	
	10.8	10.6	98	†	
	10.8	11.2	104	10.7	99
		11.0 av.	102 av.	10.75 av.	99.5 av.

\* Low result believed due to incomplete digestion (Kjeldahl).

† Used to determine melting point—135° C.

## DISCUSSION

The following points should be borne in mind in the assay:

1. The benzoyl derivative of benzedrine is somewhat volatile at 100° C. This was shown by the following experiment. A 322 mg. residue having a melting point within the range for the pure compound was heated in an oven at 100° C. for 2 hours. The weight dropped to 318 mg., a loss of 1.2 per cent. After a second heating of 3 hours at 100° C., the weight had dropped to 307 mg., a total loss of 4.7 per cent. A further heating at 100° C. for 4 hours caused a further drop in weight to 271 mg., or a total loss of 15 per cent.

2. An excess of alkali is essential to the formation of the derivative. If a large aliquot of the acid benzedrine solution is used, it may be necessary to add more sodium hydroxide solution to the reaction mixture, since the excess benzoyl chloride itself will neutralize a material amount of the alkali.

3. The accuracy of the method is dependent not only upon complete conversion of benzedrine to the benzoyl derivative, but also upon the complete hydrolysis of the excess benzoyl chloride. Therefore, if the odor of the extracted material suggests either free benzedrine base or benzoyl chloride, the results will be quantitatively valueless.

## SUMMARY

A gravimetric method for the determination of benzedrine in the absence of interfering substances was studied. Recoveries by the proposed method compare favorably with those obtained by two other available methods. The proposed method is more direct and requires fewer reagents than do the other methods considered.

It is recommended that the method described in this report be submitted for collaborative study during the coming year.

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No report on hydroxyquinoline sulfate was given by the associate referee.

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## REPORT ON METHYLENE BLUE

By H. O. MORAW (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

The official iodine volumetric method for determination of methylene blue, *Methods of Analysis, A.O.A.C.*, 1940, 576, 56, was tentatively adopted on the basis of satisfactory results by collaborators in 1923, *This Journal*, 7, 20 (1923). Two of the collaborators, Jablonski and Wales,

checked the results on the collaborative samples by the titanium trichloride method. Wales also checked them by the spectrophotometer. The next report by the associate referee, *Ibid.*, 8, 51 (1924), was confined chiefly to a study of methods for the determination of moisture in the product, and it recommended tentative adoption of drying at 110°C. and further collaborative study of both moisture determination and the iodine method. However, on the recommendation of the referee, the subject was discontinued.

In 1927, W. C. Holmes, *Ibid.*, 10, 505 (1927), submitted experimental data and references to show the influence of variations in acidity, residual iodine, and dye concentration on the iodine absorbed in the official method. He suggested that since the method is not one in which a definite atomic reaction occurs, accurate work would require calibration for variations in dye concentration. When the official method was proposed, the results indicated that under the conditions specified five atoms of iodine reacted with the molecule of methylene blue and the collaborative results at that time seemed to confirm this reaction. However, a limited amount of experimental work (see Table 1) done recently on a sample of known purity substantiates Holmes' observations but indicate a rather constant iodine absorption under the conditions outlined in the method. Further tests were also made to determine what reacting weight of pure methylene blue could be substituted for the present factor in the official method when the prevalent custom of calculating it to the moisture free basis is taken into consideration. This will necessitate a determination of moisture. The official method provides for calculating the product to methylene blue  $3\text{H}_2\text{O}$  and makes no provision for determining loss on drying. Wales and Nelson have shown that water in methylene blue is not crystal water.<sup>1</sup>

For the above work, an experimental lot was prepared by recrystallizing some methylene blue from a filtered aqueous solution of the U.S.P. medicinal product and determining its purity by its nitrogen and moisture content. The nitrogen by four determinations was 11.61, 11.61, 11.59, and 11.59 per cent—average, 11.60 per cent. The loss on drying at 110°C. for about 19 hours was 10.97, 11.03 per cent—average 11.00 per cent. From the nitrogen and moisture determinations the purity of the experimental lot was computed to 99.12 per cent. A series of determinations was made on portions of this lot by the iodine method now official, except that the first 30 ml. of the filtrate of the reaction mixture was discarded in the case of both the blank and the determinations. The results are given in Table 1.

The volumes of the reaction mixture present during the one hour of standing varied from 150 to 180 ml., as would probably occur in practical

<sup>1</sup> *J. Am. Chem. Soc.*, 45, 1657 (1923).

TABLE 1.—*Anhydrous methylene blue equivalent to 1 ml. of 0.1 N iodine*

DET. NO.	METHYLENE BLUE HYDRATED TAKEN	0.1 N I CONSUMED	ANHYDROUS METHYLENE BLUE=1 ML. 0.1 N I
	grams*	ml.	grams
h	0.1019	13.18	0.006647
k	0.1016	13.70	0.006542
l	0.1005	13.28	0.006676
		Av. of three =	0.006622
g	0.1101	14.79	0.006567
m	0.1138	15.37	0.006532
n	0.1098	14.52	0.006710
		Av. of three =	0.006603
d	0.1259	16.91	0.006568
o	0.1247	16.53	0.006655
p	0.1282	17.20	0.006575
i	0.1290	17.12	0.006647
j	0.1252	16.29	0.006780
		Av. of five =	0.006645
q	0.1406	18.94	0.006549
r	0.1394	18.59	0.006615
e	0.1503	19.91	0.006659
f	0.1493	20.10	0.006553
		Av. of four =	0.006594
		Av. of fifteen =	0.006618

\* Factor for calculating to the pure anhydrous basis =  $0.89 \times .9912 = 0.88217$ .

use. A constant relation between this volume and the amount of methylene blue would probably cause the iodine absorption to be more consistent.

It is recommended\*—

(1) That the factor in the official method for the determination of methylene blue be changed to 1 ml. 0.1 N iodine = 0.006618 gram of methylene blue anhydrous,  $C_{16}H_{18}N_2ClS$ .

(2) That paragraph 58, Chapter 39 of *Methods of Analysis, A.O.A.C.*, 1940, be changed to provide for discarding the first 30 ml. of the filtrate of the reaction mixture by adding after the word "paper" in the 8th line . . . , "discarding the first 30 ml. of the filtrate".

(3) That determination of loss on drying be provided for in XXXIX 57(a) as follows:

Foreign material absent.—Determine the loss on drying an accurately weighed portion of the sample heated at 110°C. for 12–14 hours. Weigh, and heat at the same temperature for 1 hour periods, until the loss does not exceed 1 mg. between weighings. For the determination of the methylene blue, weigh into a 50 ml. beaker 0.1–

\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 59 (1941).

0.14 gram of the prepared sample, which has not been heated above 100°C., and transfer to a 200 ml. volumetric flask with about 100 ml. of water. Dissolve completely by warming at 70–80°C. with frequent shaking during 30 minutes.

## REPORT ON AMINOPYRINE, ACETOPHENETIDIN, AND CAFFEINE

By J. CAROL (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

In accordance with the recommendations made last year, investigational work on the separation and estimation of aminopyrine, acetophenetidin, and caffeine was undertaken.

Aminopyrine, being a base, forms salts with acids. Warren<sup>1</sup> and Sinton and Rotondaro, *This Journal*, 22, 678 (1939), found that dilute sulfuric acid would completely retain aminopyrine in solution, allowing other synthetics to be separated by chloroform.

A method of analysis based on this property was suggested by Warren (private communication), and this method, with slight changes, was used in this investigation.

### *Separation of Aminopyrine, Acetophenetidin, and Caffeine in Mixtures*

*Aminopyrine.*—Transfer 2 grams of the powdered mixture into a separator, add 15 ml. of 10% (w/v) H<sub>2</sub>SO<sub>4</sub> and 50 ml. of CHCl<sub>3</sub>, and shake well. Draw off the CHCl<sub>3</sub> into a second separator and wash with 15 ml. of 10% H<sub>2</sub>SO<sub>4</sub>. Filter the CHCl<sub>3</sub> through cotton into a flask. Extract the mixture in the first separator with 5 more portions of 25 ml. each of CHCl<sub>3</sub>, washing each portion successively through the diluted H<sub>2</sub>SO<sub>4</sub> as before, filter, and collect the CHCl<sub>3</sub> in the flask. Test for complete extraction. Reserve this solution for the determination of acetophenetidin and caffeine. Add the acid washing in the second separator to the first separator. Render the mixture alkaline with ammonia T.S. and remove the aminopyrine by successive extractions with 25 ml. portions of CHCl<sub>3</sub>. Wash each CHCl<sub>3</sub> extract in a second separator with 5 ml. of water containing a few drops of ammonia T.S. and filter the solvent through cotton into a tared beaker. Evaporate the solvent. Add a few ml. of anhydrous ether\* and again evaporate. Dry the residue at 80° and weigh as aminopyrine.

### *Acetophenetidin and Caffeine*

Proceed as directed in *Methods of Analysis*, A.O.A.C., 1940, 570, 32(b).

A mixture containing aminopyrine 20 per cent, acetophenetidin 30 per cent, and caffeine 2.5 per cent q.s. lactose, was prepared and submitted to collaborators for analysis by the proposed method. The results reported by the collaborators appear in the table.

<sup>1</sup> *J. Am. Pharm. Assoc., Scientific Ed.*, 29, 115, (1940).

\* Anhydrous ether is used to facilitate the elimination of the last traces of CHCl<sub>3</sub>. Vanderkleed and E' We have shown that certain organic residues, after evaporation of CHCl<sub>3</sub>, persistently retain traces of the solvent after drying at 80°, *J. Am. Pharm. Assoc.*, 5, 713 (1916).



COLLABORATOR	AMINOPYRINE		ACETOPHENETIDIN		CAFFEINE	
	FOUND	RECOVERY	FOUND	RECOVERY	FOUND	RECOVERY
R. Hyatt	19.85	99.3	29.37	97.9	2.46	98.4
	19.71	98.5	29.60	98.7	2.54	101.6
	19.95	99.8	29.62	98.7	2.54	101.6
E. H. Berry	19.90	99.5	29.23	97.4	2.53	101.2
	19.89	99.5	29.53	98.4	2.50	100.0
H. R. Bond	19.54	97.7	29.78	99.3	2.46	98.4
	19.64	98.2	29.73	99.1	2.61	104.4
J. Carol	20.03	100.2	29.87	99.6	2.48	99.2
	20.19	100.9	29.92	99.7	2.55	102.0
	20.13	100.7	30.10	100.3	2.46	98.4
G. M. Johnson	19.73	98.7				
	19.73	98.7				
L. E. Warren	20.01	100.0	30.45	101.5	2.58	103.2
	20.06	100.3	lost	lost	lost	lost
	20.23	101.1	30.06	100.2	2.58	103.2
	19.80	99.0	29.71	99.0	2.62	104.8
	19.92	99.6	29.93	99.7	2.73	109.2
	19.37	96.9	30.45	101.5	2.70	108.0
H. H. Shull	20.06	100.3	30.16	100.5	2.64	105.6
	20.07	100.4	30.41	101.3	2.57	102.8
Average	19.89	99.5	29.87	99.9	2.56	102.4

The results for aminopyrine are considered satisfactory for a mixture of this type. The results for acetophenetidin and caffeine are comparable with those obtained by Grove, *This Journal*, 22, 723 (1939).

It is recommended\* that the procedure presented for the separation of aminopyrine from acetophenetidin and caffeine and its determination be accepted as a tentative method.

No report on ethyl aminobenzoate was given by the associate referee.

## REPORT ON SULFAPYRIDINE

By IMAN SCHURMAN (U. S. Food and Drug Administration,  
Cincinnati, Ohio), *Associate Referee*

During the past few years many *N*-substituted sulfanilamides have been synthesized. Perhaps the one receiving the most attention at the present

\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 52 (1941).

time is sulfapyridine (2-sulfanilamidopyridine), due to its wide publicity in the treatment of pneumonia.<sup>1</sup>

Although the literature is replete with references to the pharmacological studies of the drug, little information relating to its chemistry<sup>2</sup> had been published previous to the appearance of an article by Crossley, Northey and Hultquist, entitled "Constitution and Properties of 2-Sulfanilamidopyridine."<sup>3</sup> In this article the physical and chemical properties of the carefully purified compound are given in detail, and mention is also made that the compound may be quantitatively determined by diazotization with a standard sodium nitrite solution.

In New and Nonofficial Remedies (1940) the compound is also assayed by the nitrite method. In addition many colorimetric methods have been proposed, but these are generally applied to the determination of the drug in body fluids.

For the purpose of this report, the nitrite method was investigated. After some preliminary work the following proposed method and notes, together with two samples of sulfapyridine, were submitted to several collaborators.

#### REAGENTS

(1) *Sodium nitrite solution*.—0.1 *N*. Dissolve 7 grams of  $\text{NaNO}_2$  in water and dilute to 1 liter. Standardize against pure sulfanilic acid (recrystallized twice from water and dried at 100°C. to constant weight). Weigh accurately ca. 0.35 gram of sulfanilic acid into a 150 ml. beaker, add 50 ml. of water and 5 ml. of  $\text{HCl}$ , and heat until the sample is dissolved. Cool to 15°–20°C. and titrate with the  $\text{NaNO}_2$  solution. Stir the solution with a 3 mm. glass rod for ca. 1 minute after the addition of each drop of nitrite solution before testing for the end point. The end point is an immediate blue streak when the glass rod is rapidly drawn through a smear of starch-iodide paste on a filter paper.

1 ml. of 0.1 *N*  $\text{NaNO}_2$  = 0.01732 gram of sulfanilic acid.

(2) *Starch-iodide paste*.—Bring 430 ml. of water to a boil in a beaker set in an oil bath and add 3 grams of  $\text{KI}$  dissolved in 15 ml. of water, followed by 7.5 grams of  $\text{ZnCl}_2$  dissolved in 30 ml. of water. While the solution is boiling, gradually add a slurry of 20 grams of potato starch in 100 ml. of cold water, with agitation. Boil for 2 minutes, then cool and store in tightly stoppered bottles.

#### DETERMINATION

*Sample A*.—Weigh accurately 0.5 gram of the powder and transfer to a 150 ml. beaker. Add 50 ml. of distilled water and 5 ml. of conc.  $\text{HCl}$ . Cool to 15°–20°C. and titrate with 0.1 *N*  $\text{NaNO}_2$  (ca. 18 ml. of the nitrite may be added rapidly). Dip a 3 mm. glass rod into the solution after each addition of nitrite and draw rapidly through a smear of starch-iodide paste on No. 1 Whatman paper or its equivalent. The end point is an *immediate* blue streak, which should be permanent for 1 minute or longer.

1 ml. of 0.1 *N*  $\text{NaNO}_2$  = 0.02493 gram of sulfapyridine.

*Sample B*.—Weigh accurately 0.7 gram of powder, transfer to a filter paper, and extract with acetone. (Usually 100 ml. of acetone is sufficient for complete extrac-

<sup>1</sup> Whitby, *Lancet*, 1, 1210 (1938).

<sup>2</sup> Marshall, *J. Am. Med. Assoc.*, 112, 352 (1939).

<sup>3</sup> *J. Am. Chem. Soc.*, 62, 372 (1940).

tion.) Evaporate the acetone on a steam bath with the aid of an electric fan. To the dry residue add 50 ml. of distilled water and continue as directed under *Sample A*.

#### NOTES

1.—It is important that temperature of diazotization be observed because at low temperature the diazotization is slow.

2.—Starch-iodide paste is more reliable than starch-iodide paper.

3.—The starch-iodide paste must give a definite blue color streak when a glass rod dipped into a solution of 1 ml. of 0.1 *N* NaNO<sub>2</sub> and 10 ml. of concentrated HCl in 1 liter of distilled water is drawn across a smear of the paste. If not, fresh paste must be prepared.

Each collaborator was requested to report per cent sulfapyridine in Samples A and B and the method used for standardizing the sodium nitrite solution.

Samples A and B were prepared as follows:

*Sample A*.—Specially purified sulfapyridine, M.P. 191°–192°C.; nitrogen by Kjeldahl 16.73% (Theor. 16.86%).

*Sample B*.—Sulfapyridine, 70%; starch 15%; lactose 15%. The sample was thoroughly mixed by being passed through a 60-mesh screen several times. Per cent sulfapyridine: by nitrogen determination, 69.93; by acetone extraction and drying residue at 100°C., 69.94 and 70.03.

The results obtained by the Associate Referee and the collaborators are shown in Table 1.

#### COMMENTS OF COLLABORATORS

*Robert L. Herd*.—The dry extraction should be made by transferring the sample to a small beaker, adding the acetone, warming on steam bath with stirring, and filtering.

It was found that better results were obtained when standardizing the sodium nitrite solution with sulfanilic acid when the solution was stirred for about one-half minute before the end point was tested. This insures the complete reaction of the HNO<sub>2</sub>, which in some instances might give a false end point.

*J. H. Cannon*.—The end point is not so good on the sulfapyridine as on the sulfanilic acid on account of the yellow color developed by the former.

*S. B. Falck*.—The amount of sample taken for analysis gives a titer of only approximately 20 ml. Would it not be better to double the size of sample to yield approximately 1 gram of sulfapyridine, which would have a titer of approximately 40 ml?

*H. R. Bond*.—Results listed under (1) and (2) were obtained at room temperature (about 25°C.). Those under (3) were obtained at 15°–20°C. Under (1) the end point was obtained by the use of the iodo-starch indicator on filter paper, under (2) and (3), by the use of indicator on spot plate.

*H. Rogavitz*.—I suggest rewording the sentence, "The end point is an *immediate* blue streak, which should be permanent for 1 minute or longer." The blue streak, when once obtained, remains permanent. I assume that you mean the end point is permanent for 1 minute or longer—indicating that excess nitrite is present.

I had difficulty in interpreting what the true end point was, especially in the case of sulfapyridine, where the yellow solution obscures the blue streak. I notice that New and Non-Official Remedies determines sulfapyridine and sulfanilamide in tablets without removing the excipients. Are there any excipients that might

TABLE 1.—*Collaborative results*

COLLABORATORS (ALL MEMBERS OF THE FOOD AND DRUG ADM.)	SAMPLE		0.1 N NaNO <sub>2</sub> STANDARDIZED AGAINST—
	A	B	
	<i>per cent</i>	<i>per cent</i>	
I. Schurman	100.3	70.0	Sulfanilic acid
Cincinnati	100.3	69.9	
	100.2	69.9	
S. B. Falck	100.7	69.3	Sulfanilic acid
Cincinnati	100.7	69.3	
J. H. Cannon	100.0	68.8	Sulfanilic acid
St. Louis			
Robert L. Herd	100.4	70.0	Sulfanilic acid
Washington	100.2	70.0	
Harold F. O'Keefe	99.96	68.24	Sulfanilic acid
Chicago	100.11	68.21	
	100.01	68.32	
	99.96	68.19	
	100.11	68.29	
	100.01	68.32	
Granville Q. Lipscomb	98.63	68.17	U.S.P.XI Assay for nitrites
Baltimore	98.63	67.99	
	97.87	67.46	
	100.36	67.46	
	100.50		
G. Ivor Jones	99.62	67.16	Sulfapyridine
San Francisco			
H. R. Bond	(1) 100.10	(1) 70.45	U.S.P.XI Assay for Nitrites
Kansas City	100.10		
	(2) 100.10	(2) 70.45	
		70.45	
	(3) 100.10		
	100.10		
H. Rogavitz	101.1	70.9	Sulfanilic acid
New York	100.8	70.9	
	100.0	70.8	

interfere with the diazotization titration? If an acetone extraction is used, could not the residue be dried and weighed for a rough check on the volumetric determination?

The normality of the sodium nitrite solution, standardized against desiccated sulfanilic acid was 0.1008. The solution was also standardized against *p*-amino-

benzene sulfonylamide (Eastman), by the proposed method. (The crystals were not desiccated and no  $\text{NH}_4\text{OH}$  was used to dissolve the crystals.) The normality thus obtained was 0.1005. The solution was also standardized by the U.S.P. XI method for the assay of sodium nitrite and the normality was found to be 0.1016.

The purity and degree of hydration of the sulfanilic acid cannot be quickly determined. Sulfanilamide or sulfapyridine, if available, might be a preferable primary standard.

#### DISCUSSION OF RESULTS

The results on Sample A are quite satisfactory, particularly the ones where the nitrite solution was standardized against sulfanilic acid.

Although satisfactory results were obtained for Sample B in certain determinations, the results as a whole show considerable variation. This may be due to incomplete extraction of sulfapyridine from the sample, or to the fact that the nitrite solution was not standardized against sulfanilic acid.

It is recommended\* that the study be continued with a view to the development of suitable qualitative tests that would be specific for sulfapyridine, and to the development of new quantitative methods.

The Associate Referee wishes to express his appreciation to the collaborators for their fine cooperation and especially to E. H. Northey of the Calco Chemical Company for furnishing the purified sulfapyridine.

#### REPORT ON VEGETABLE DRUGS AND THEIR DERIVATIVES

By FRANK H. WILEY (U. S. Food and Drug Administration,  
Federal Security Agency, Washington, D. C.), *Referee*

The seven associate referees assigned to this field have indicated that the problems are in various degrees of completion. One report is recommended for tentative acceptance, three reports are submitted with recommendation for further study, and three associates submitted no report but expressed the desire to continue the problems another year.

*Physostigmine Salicylate*.—The associate referee last year reported a method with which he obtained good results. This method, however, did not prove so reliable in the hands of the collaborators. It was recommended that the work be continued another year. Results obtained in the past year have been more uniform, and the associate referee recommends tentative acceptance of the method. The Referee concurs. It is also recommended that a study be made of the application of the method to ointments containing physostigmine salicylate.

*Arecoline Hydrobromide*.—The associate referee reports that he has devised a method for the assay of this material based on extraction with a volatile solvent and subsequent titration. It is recommended by the as-

\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 53 (1941).

sociate referee and the Referee that this method be subjected to collaborative study.

*Phenobarbital and Theobromine.*—The associate referee has submitted a report on this project, together with the results of collaborative investigations. The results, in the opinion of the associate, do not justify its tentative adoption and he recommends that this study be continued another year. The Referee concurs.

*Chemical Methods for Ergot Alkaloids.*—The associate referee was handicapped in obtaining pure samples of the ergot alkaloids due to war conditions in Europe. The materials have now been obtained, and it is recommended that this project be continued.

*Theophyllin Sodium Salicylate.*—The associate referee has revised the method reported last year, but owing to the burden of official duties he was unable to submit samples for collaborative investigation. He and the Referee recommend the continuation of this project.

*Quinine Ethyl Carbonate.*—The associate referee, owing to official duties, was unable to devote any time to this problem. He expresses the desire that it be reassigned to him for another year, and the Referee so recommends.

*Plasmochin.*—The associate referee had difficulty in securing a pure sample of plasmochin until it was too late to make this study. While he hopes to make a report soon, the Referee would recommend that this subject be continued unless a final report is forthcoming before the next meeting of the Association.

Since only one of the studies assigned last year is being recommended for tentative acceptance, and since it is further recommended that this work be extended to other preparations containing this material, no new subjects are suggested for consideration.

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No report on chemical methods for ergot alkaloids was given by the associate referee.

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No report on theophylline sodium salicylate was given by the associate referee.

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## REPORT ON PHYSOSTIGMINE SALICYLATE

By GEORGE M. JOHNSON (U. S. Food and Drug Administration,  
St. Louis, Mo.), *Associate Referee*

The method for the determination of physostigmine was submitted to further collaborative study this year. The collaborative work reported last year gave somewhat low recovery of the alkaloid present in the collaborative sample. A new sample was prepared and submitted to the collaborators with the revised method, which is essentially that previously

given except that it has been elaborated to prevent ambiguity. The collaborative sample was a mixture of lactose and 2.27 per cent by weight of physostigmine salicylate. Purity of the physostigmine salicylate based on the nitrogen content was 97.7 per cent. (That used last year had a different control number and assayed 100 per cent.) Extraction of the alkaloid and titration gave 97.1 per cent. The method used by the collaborators is as follows:

#### PREPARATION OF SAMPLE

Count and weigh a representative number of tablets and calculate the average weight. Grind to fine powder in a mortar.

#### DETERMINATION

Weigh accurately a sufficient quantity of the powdered material to contain ca. 1 grain of physostigmine salicylate, transfer to a separator, and add enough water (not exceeding 20 ml.) to dissolve the material. Make alkaline to litmus with solid  $\text{NaHCO}_3$  and extract to completion at once with  $\text{CHCl}_3$  (usually 30, 20, 20, 10, and 10 ml. portions are sufficient). Transfer each extract to a second funnel containing 5 ml. of water. Wash each extract with this 5 ml. of water and filter into a beaker, using in the stem of the funnel a cotton pledget moistened with  $\text{CHCl}_3$ . Test for complete extraction by making an extra extraction with a 5 ml. portion of  $\text{CHCl}_3$  and treat separately as directed below. Evaporate combined  $\text{CHCl}_3$  extracts on a water bath, using a current of air to assist evaporation. When the volume has been reduced to ca. 5 ml., remove from bath and complete evaporation without aid of heat. Dissolve residue in a few ml. of neutral alcohol. Add an excess of 0.02  $N$   $\text{H}_2\text{SO}_4$ . Cover with a watch-glass and heat on steam bath until all alkaloids have been washed down the sides by the refluxing action. Remove the watch-glass and evaporate the bulk of the alcohol. Cool. Add methyl red indicator and titrate excess acid with 0.02  $N$   $\text{NaOH}$ . Calculate the equivalent of physostigmine salicylate. 1 ml. of 0.02  $N$   $\text{H}_2\text{SO}_4$  = 0.00826 gram of physostigmine salicylate.

COLLABORATOR	PHYSOSTIGMINE SALICYLATE FOUND	RECOVERY BASED ON 97.7% PURITY
	<i>per cent</i>	<i>per cent</i>
J. P. Aumer New Orleans	2.19	98.7
	2.20	99.2
E. H. Berry Chicago	2.12	95.6
	2.14	96.5
S. D. Fine St. Louis	2.14	96.5
	2.19	98.7
	2.24	101.0
H. F. O'Keefe Chicago	2.15	96.9
I. S. Schurman Cincinnati	2.16	97.4
	2.18	98.3

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N. H. Shull	2.08	93.8
McNeil Lab.	2.16	97.4
Philadelphia		
J. B. Snider	2.16	97.4
Minneapolis		
G. M. Johnson	2.13	96.0
St. Louis	2.15	96.9
	2.17	97.8
	2.19	98.7
Average	2.16	97.5

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The average recovery of the alkaloidal salt is 97.5 per cent, which is somewhat better than that of last year, namely, 96.1 per cent. This is still less than might be desired. However, in view of the larger number of collaborators and the somewhat improved recovery, together with the small amount of material involved and the consequent small titration, the associate referee considers that the method is acceptable. Moreover, the method is a general one for alkaloids and no indications of decomposition were noted.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method of assay for physostigmine salicylate be accepted tentatively.

(2) That the assay of physostigmine in ointments and other mixtures be studied.

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#### REPORT ON ARECOLINE HYDROBROMIDE

By HENRY R. BOND (U. S. Food and Drug Administration,  
Kansas City, Mo.), *Associate Referee*

A method of assay for arecoline hydrobromide was devised this year. It utilizes the general principles of extraction with a volatile solvent from an alkaline solution with subsequent titration of the alkaloid. No extensive collaborative work was completed, due to difficulties involving in particular the purity of the material to be assayed and the proper organic solvent to be used.

After several recrystallizations from alcohol, the arecoline hydrobromide was found to be reasonably pure by melting point determination and by assay for bromide content, which indicated a purity of 99.03 per cent.

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\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 53 (1941).



The method employed for the extraction of arecoline and titration of the alkaloid is as follows:

Accurately weight a sample of sufficient size to insure the presence of 0.1–0.15 gram of arecoline hydrobromide. Rinse the sample into a 125 ml. separatory funnel, the stem of which holds a fairly tight-fitting cotton pledget moistened with  $\text{CHCl}_3$ . Add enough water to the contents of the separator to make a volume of 20 ml., an amount sufficient to dissolve the arecoline hydrobromide; to the solution add solid  $\text{NaHCO}_3$  until a small quantity of the bicarbonate remains undissolved.

Extract with  $\text{CHCl}_3$ , using 30, 25, 20, 15, 10 ml. portions, and test for complete extraction. Draw off extract into a 250 ml. tall form beaker or preferably a 500 ml. Erlenmeyer flask. To the beaker or flask add 35 ml. of 0.02  $N$   $\text{H}_2\text{SO}_4$ , and evaporate the  $\text{CHCl}_3$  on the steam bath, using glass beads or a stirring rod to prevent superheating. When the  $\text{CHCl}_3$  is evaporated, cool the acid solution and titrate with 0.02  $N$   $\text{NaOH}$  solution, using 2 drops of methyl red indicator. 1 ml. of 0.02  $N$  acid = 0.00472 gram of arecoline hydrobromide.

Determined by the method outlined, the quantity of arecoline extracted was equivalent to 98 per cent of the weight of sample or 99 per cent of the arecoline hydrobromide as assayed for bromide content.

It is recommended that collaborative study be made of the assay method presented.

No report on quinine ethyl carbonate was given by the associate referee.

## REPORT ON PHENOBARBITAL AND THEOBROMINE

By E. C. DEAL (U. S. Food and Drug Administration,  
New Orleans, La.), *Associate Referee*

Phenobarbital is official in U. S. Pharmacopoeia XI, but no method of assay is given. The sodium salt is also listed in the same compendium together with an assay based upon extraction with ether, after acidification with hydrochloric acid, and subsequent weighing of the dried extractive. A similar procedure is given in *Methods of Analysis, A.O.A.C.*, 1935, for the determination of phenobarbital by extracting with chloroform-ether solvent. Budde<sup>1</sup> titrates phenobarbital with silver nitrate after dissolving in sodium carbonate solution. Some work has been done by Payne, *This Journal*, 21, 566 (1938), on the feasibility of titrating the substance with sodium hydroxide according to the method outlined by Morin<sup>2</sup> and by Babich,<sup>3</sup> using thymolphthalein as indicator.

Theobromine in combination with sodium salicylate is official in U.S.P. XI. The empirical assay given depends upon the direct weighing of the precipitated theobromine upon neutralization of the sodium salicylate, allowance being made for the solubility of the theobromine. It is obvious that such a method could not be used as a general procedure.

<sup>1</sup> *Apoth. Ztg.*, 49, 295 (1934); *Chem. Abstracts*, 28, 3176 (1934).

<sup>2</sup> *J. Pharm. Chim.*, 21, 69 (1935).

<sup>3</sup> *Pharm. Monatsch.*, 17, 87 (1936).

The present problem is concerned with the isolation and determination of phenobarbital and theobromine in mixtures of the two substances. Since these compounds are usually dispensed in tablet form with starch, lactose, or other excipients, any useful method must take cognizance of these diluents. Combinations of these two drugs as sold on the market are usually in the ratio of one part of phenobarbital to ten parts of theobromine. No accurate methods were found for the separation and determination of the two substances when occurring together. The A.O.A.C. method for phenobarbital gives results 10–20 per cent high in the presence of theobromine due to the partial extraction of this substance. The error is decreased to about 5 per cent by using water-washed ether instead of the chloroform-ether mixture as the solvent. Moraw has worked out a somewhat empirical method (unpublished) by limiting the volume of ether used for the extraction.

A search of the literature revealed three general methods for determining theobromine quantitatively: (a) Kockum<sup>4</sup> argentometric method. A modification of this method was applied by Miko<sup>5</sup> for the analysis of theobromine-sodium salicylate. Boic<sup>6</sup> titrates the acid liberated when a neutral solution of silver nitrate is added to theobromine. (b) Self and Rankin<sup>7</sup> converted theobromine into caffeine by methylating with dimethyl-sulfate, and determined the caffeine by extracting with an organic solvent and then weighing. This method is official in the British Pharmacopoeia. (c) Emery-Spencer<sup>8</sup> iodometric procedure. This method was adopted by this Association, *This Journal*, 19, 105 (1936), for the assay of theobromine-calcium. Breukeleven<sup>9</sup> modified it by using normal sodium hydroxide instead of glacial acetic acid as the initial solvent.

In the application of these methods to the present problem, the quantitative results have been disappointing. The Associate Referee found that phenobarbital can be separated quantitatively from theobromine by using water-washed ether for extracting the phenobarbital and then extracting this ether solution with a portion of 10 per cent hydrochloric acid to remove small amounts of theobromine that are extracted by the ether. This procedure gives useful results in the hands of the Associate Referee.

Because of mechanical and solubility difficulties the Associate Referee has been unable to devise a process for measuring the theobromine after removal of the phenobarbital. The relative insolubility of theobromine in the known organic solvents has made quite difficult the problem of its separation from excipients, which are also insoluble in these solvents. The Kockum argentometric method could not be applied directly to mixtures of theobromine and phenobarbital due to the reaction of the silver nitrate

<sup>4</sup> *Svensk Farm. Tid.*, 12, 81 (1908); *Apoth. Ztg.*, 23, 230 (1908).

<sup>5</sup> *Pharm. Monatsh.*, 14, 279 (1933); *Chem. Abstracts* 28, 1811 (1934).

<sup>6</sup> *Pharm. Ztg.*, 75, 988 (1930); *Chem. Abstracts* 25, 169 (1931).

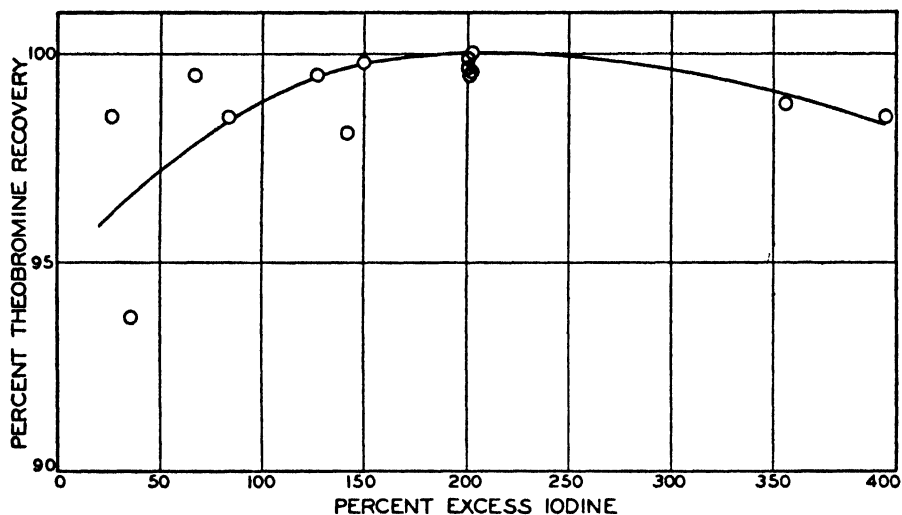
<sup>7</sup> *Quar. J. Pharm. Pharmacol.*, 4, 436 (1931).

<sup>8</sup> *J. Ind. Eng. Chem.*, 10, 605 (1918).

<sup>9</sup> *Chem. Weekblad*, 24, 206 (1927).

with phenobarbital. Likewise, the Self-Rankin method proved to be inapplicable since a methylation product soluble in organic solvents is formed from the phenobarbital during the methylation of the theobromine. The Emery-Spencer method as modified by Breukeleven was thought to offer the best solution of the problem.

Preliminary experiments showed that the presence of phenobarbital had no effect whatever on the iodometric determination of the theobromine. Consequently, it is not necessary to remove this substance. However, incomplete recoveries were obtained by the method as published when pure theobromine was used, with or without added phenobarbital. A search



for the cause of the shortages showed that the recoveries of theobromine are proportional to the quantity of iodine solution used until this excess amounts to about 130 per cent. From this point on until about 200 per cent excess was added quantitative recoveries were obtained. When an excess of 200 per cent was added the recoveries diminished, probably due to a solubility factor affecting such a small sample. This phenomenon is graphically shown in the chart. In the case of theophyllin assayed by essentially the same procedure a similar conclusion was reached by Reimers,<sup>10</sup> who found that approximately 150 per cent excess of iodine was required for its quantitative recovery.

While substances such as starch and dextrin, which are known to absorb iodine, under the conditions of the determination might be expected to interfere, surprisingly lactose was found also to influence the results. Due to its solubility in water, lactose might be removed by washing, but the problem of removing starch has not yet been solved. So far, attempts to

<sup>10</sup> *Danak Trade. Farm.*, 9, 11 (1935).

hydrolyze the starch by refluxing with hydrochloric acid have not proved satisfactory.

The results of the collaborative studies are too much at variance to merit inclusion in this report. It is, accordingly, recommended that the subject be further studied.

## REPORT ON PLASMOCHIN

By F. C. SINTON (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

Plasmochin is a basic compound of synthetic origin and its chemical formula may be expressed as 8-( $\delta$ -diethylamino- $\alpha$ -methylbutylamino)-6-methoxyquinoline.

This product has found use as an antimalarial remedy and is commercially available in tablets as a plasmochin salt. Plasmochin is also used for the same purpose in conjunction with quinine. The two may be encountered together in tablets. The work reported here was conducted to develop a method for plasmochin alone, and also for plasmochin and quinine in mixture.

For purposes of experiment a sample of plasmochin base was obtained through cooperation of the manufacturer. This sample consisted of a greenish, viscous, oily liquid contained in a dark colored glass-stoppered bottle. Description of plasmochin in the U. S. Dispensatory and Merck's Index as a powder presumably refers to one of the salts rather than the base.

Determinations made on the sample of plasmochin included nitrogen assay, acid titration, titration with sodium nitrite solution, and non-volatile matter.

Recoveries were as follows:

	<i>per cent</i>	
Non-volatile matter, 1 hr. at 100°C.	99.4	
Nitrogen calculated to plasmochin	99.5	
Titration with standard acid	99.6	99.9
Titration with sodium nitrite solution	99.3	99.2

These results indicate the material to be of satisfactory purity and, furthermore, that any one or a combination of the methods could likely be used in the determination of plasmochin. In the case of the non-volatile matter a darkening was observed indicating some decomposition but an acid titration on the dried material showed a difference of only 0.4 per cent. Titration with acid was in the usual manner as in the case of alkaloids, an excess of standard acid being titrated with 0.02 *N* alkali. The acid solution of plasmochin has a rather deep yellow color, which lightens as alkali is added near the end point. However, methyl red was found to be rather unsatisfactory as an indicator, since it was difficult to see a definite

change from a reddish yellow to yellow. Bromocresol purple was found satisfactory in its color change and was used in the above and subsequent acid titrations. It was further found that the color change of bromocresol purple gave slightly higher results but was quite close to the end point obtained by a potentiometric titration. The color change with bromocresol purple was at *pH* 6, whereas the point of inflection on the potentiometric curve was at *pH* 6.5. The difference in titration amounted to somewhat less than 0.1 ml. of 0.02 *N* alkali.

In order to determine how accurately plasmochin can be extracted, a solution was prepared by weighing a known amount and adding ether, followed by an excess of acid. The ether was evaporated and the acid solution made to volume with water. Samples equivalent to about 0.1 gram were transferred to separatory funnels, made alkaline with ammonia, and extracted completely with chloroform.

The chloroform was evaporated to about 5 ml. and after an excess of standard acid had been added the residual chloroform was evaporated and the excess acid titrated with 0.02 *N* alkali.

As a check determination the titrated material was made acid with a few drops of hydrochloric acid, the solution cooled with ice, and then titrated with 0.1 *N* sodium nitrite solution, starch-potassium iodide paper being used as an indicator.

Recoveries obtained were as follows:

<i>Method</i>	<i>per cent</i>		
Acid titration	99.3	99.1	99.3
Sodium nitrite titration	99.1	98.8	99.2

In the titration with nitrite it was observed that it was necessary to stir thoroughly to allow complete reaction. It was also noted that it is useless to attempt to assay a solution of plasmochin in acid that has stood around for several hours. Decomposition takes place and the color extracted obscures the indicator change. Both assays are accomplished without difficulty and appear to be quite accurate.

Further experiments were conducted with a view to finding a procedure for determining plasmochin in the presence of quinine. Precipitation of the quinine with chromate was found impractical owing to the large solubility factor. It was found, however, that quinine does not react with sodium nitrite. Since plasmochin can be titrated with nitrite this offered a feasible means of determination in case of a mixture of the two.

A solution containing known amounts of plasmochin and quinine was prepared. An aliquot was transferred to a separator, made alkaline, and extracted completely with chloroform. The chloroform was evaporated to about 5 ml., an excess of standard acid was added, and the residual chloroform was removed on the steam bath and the excess acid titrated with 0.02 *N* alkali. A gradual change in color was observed but no satisfactory end

point could be obtained. Even a potentiometric titration curve showed no marked rise at any point. Titration of the solution with sodium nitrite did, however, show good recoveries of plasmochin, amounting to 98.9 and 99.1 per cent.

Since the acid titration was not satisfactory, it was thought advisable to make a gravimetric determination of the plasmochin and quinine, after which the plasmochin could be titrated with sodium nitrite and the quinine could be determined by difference.

The solution containing the mixture of plasmochin and quinine was extracted with chloroform in the usual manner and the extracted residue was weighed after being dried for 1 hour at 100°C. The residue was dissolved in several ml. of chloroform, and after an excess of acid had been added the chloroform was evaporated and the solution titrated with sodium nitrite solution. After the determined amount of plasmochin had been deducted, the quinine was calculated by difference:

Recoveries were as follows:

	<i>per cent</i>	
Plasmochin	99.5	99.2
Quinine	100.7	101.1

#### CONCLUSIONS

Experimental work has shown that plasmochin can be satisfactorily determined by the usual shakeout method, and titrated with standard acid. The same solution can be checked by a titration with sodium nitrite solution.

Plasmochin in mixture with quinine can be satisfactorily determined by titration with standard sodium nitrite solution. This is possible since quinine does not react with sodium nitrite. In the case of such a mixture, both ingredients can be determined by a combination of gravimetric procedure and nitrite titration, the quinine being calculated by difference.

It is recommended that this topic be continued for collaborative study of methods for plasmochin and mixtures of plasmochin and quinine.

#### REPORT ON DRUG BIOASSAYS

By LLOYD C. MILLER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

In general, problems of biological assay do not lend themselves so well to collaborative study as do the problems of chemical assay. Furthermore, few members of the A.O.A.C. have the necessary equipment for drug bioassays, whereas chemical laboratory apparatus is universally available. It would seem wise, therefore, to suggest that the collaborative effort of this Association be directed toward perfecting those phases of drug bioassay methods that are essentially chemical in nature. The Referee on

Drug Bioassays recommends two subjects for collaborative study, one of which is sufficiently worked out for immediate attention.

### 1. PREPARATION OF TEST DILUTIONS OF TINCTURES

In the assays of tinctures of potent drugs, such as aconite and digitalis, the official assay methods require dilution of the tinctures prior to their injection into the test animals to eliminate undue influence of the alcohol of the vehicle upon the activity of the drug principle. Since it has been reported that variations in even relatively low concentrations of alcohol may affect greatly the activity of digitalis, it is desirable that test dilutions of both the standard and unknown samples of digitalis have essentially identical alcohol concentrations. It seems advisable also, on the grounds of good technic, to dilute so that the total volume of injected material is the same for both standard and sample. In a regulatory laboratory, where samples varying widely in potency may be encountered, it is imperative that a means be worked out for meeting these requirements. To fulfill this need, a procedure has been developed in the Division of Pharmacology of the Food and Drug Administration for preparing suitable volumes of test dilutions of tinctures of digitalis containing practically any desired alcohol concentration. In routine assays, however, all tinctures are so diluted that the dose per gram of frog, of the test material, whether standard or sample, is represented by 0.02 ml. of a fluid containing 23 per cent alcohol. This practice combines the maximum in convenience with a margin of safety below the limit of 25 per cent alcohol fixed by the U.S.P. assay directions.

On the average, tinctures of digitalis contain about 70 per cent alcohol, so that only the dilution of 3.00 ml. of tincture to 10.0 ml. with distilled water will yield a final alcohol concentration of about 23 per cent. Where the desired dose is less, so that the necessary dilution is greater than 3:10, some alcohol must be added; where, as often is necessary, more of the test material must be used, some alcohol must be removed by evaporation. While the quantity of alcohol to be added can be readily calculated, there is no available evidence concerning tinctures of digitalis to indicate the appropriate amount of evaporation. It was found possible experimentally to relate the amount of alcohol remaining in the residue left after evaporating part of a sample of tincture with the percentage of its original weight removed. This relationship is linear as shown in Figure 1. In this figure the three points corresponding to 0, 25, and 50 per cent removal are the average of 10 closely-agreeing determinations. Earlier work had indicated that above 50 per cent the straight-line relationship begins to fail. However, a single run made at 60 per cent removal was practically on the straight line.

With this information it was possible to devise a procedure that involves measuring a sample of the tincture into a tared 50 ml. beaker and determining the net weight of the sample to the nearest fiftieth of a gram.

The beaker and contents are then placed under a stream of air. From time to time the sample is reweighed until the required weight has been reached.

The evaporation was carried out under a variety of conditions with respect to temperature, size of sample, and rate of evaporation, but the only

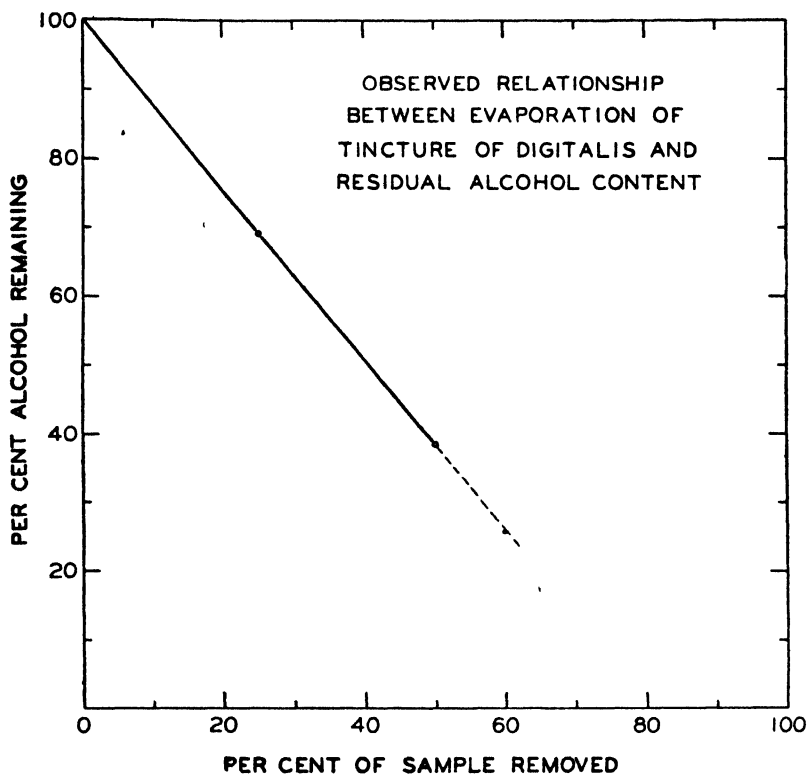


FIG. 1.

factor producing an appreciable influence was the relative humidity of the atmosphere. Thus it was found necessary to use a rather special type of nozzle to prevent drawing room air into contact with the surface of the tincture by convection currents. This precaution was essential since the moisture of humid summer air tended to condense on the cool evaporating surface so that the loss in weight was less than the amount actually evaporated. With a nozzle made of 6 mm. glass tubing protruding about 1 mm. from the center of the large end of medium-sized cork, the amount of room air drawn in is negligible. This arrangement gave uniform results, so that a table was calculated, a part of which is shown in Table 1. The procedure has been in use for the past 12 months and has proven satisfactory, but before recommending its general adoption it should be put to collaborative test. This course seems advisable also in view of the possi-



TABLE 1.—*Preparation of test dilutions of tincture of digitalis*

LOG DOSE	DOSE PER KG. OF FROG	VOL. OF ASSAY PREPN PER 10 ML. OF TEST DILUTION	MAKE TO 10 ML. VOLUME WITH SPECIAL DILUENT CONTAINING THE VOLUME INDICATED BELOW OF U.S.P. XI DIGITALIS MENSTRUUM Q.S. 100 ML. WITH DISTILLED WATER
	ml.	ml.	
.40	2.51	1.26	19.64
.50	3.16	1.58	16.64
.60	3.98	1.99	12.51
.70	5.01	2.51	6.67
Test dilutions of ca. 23% alcohol will result if weight of original sample is reduced under an air stream by percentage indi- cated below and residue is diluted to 10 ml. with water.			
.80	6.31	3.16	4
.90	7.94	3.97	18
1.00	10.00	5.00	29
1.10	12.59	6.30	38
1.20	15.85	7.92	45

bility that the procedure, if available, may find use as an initial step in chemical assays of alcohol containing pharmaceuticals.

## 2. ENTERIC COATINGS

A second general problem that ultimately must involve biological testing is that of the efficacy of enteric coatings on capsules, pills, and tablets. As defined by custom, an enteric coating is of such a nature that, following oral administration, it resists disintegration in the stomach but readily breaks down in the intestine. Several samples of products alleged to possess an enteric coating have been examined in this laboratory by exposing them to solutions of varying degrees of acidity as well as to artificial gastric juice. The data obtained so far are only preliminary, but they are sufficiently conclusive to suggest the existence of a significant regulatory problem in that 6 of the 8 products tested appear to offer little, if any, protection to their contents against gastric juice. These preliminary tests have involved exposing tablets, two at a time, to each of four different media held at body temperature. The media were water, artificial gastric juice, a citric acid-phosphate buffer solution at pH 1, and a citric acid-phosphate buffer solution at pH 8. The artificial gastric juice contained pepsin and the chlorides of sodium, potassium and calcium, and was acidified to about pH 1.75 with hydrochloric acid, which is similar to a solution that has been used considerably in development research on enteric preparations. The results are quite consistent, as will be seen from Table 2, which summarizes the findings of several tests. If it is maintained that these tablets may remain in the stomach for as long as 3 hours after ingestion, it

TABLE 2.—*In vitro* tests of commercial enteric coatings

SAMPLE	STABILITY IN ACID MEDIUM (pH 1)		DISINTEGRATION IN ALKALINE MEDIUM (pH 8)	
	3 HOURS OR MORE	1 HOUR OR LESS	2 HOURS OR MORE	LESS THAN 2 HOURS
A	0	10	0	4
B	0	12	0	6
C	0	12	0	8
D	0	10	0	6
E	0	8	0	4
F	12	0	0	6
G	12	0	0	6

seems reasonable to expect them to withstand an acid medium for that long. It would also seem reasonable to expect such tablets to disintegrate in a medium representing intestinal contents in not much over an hour. Judged by these criteria, 5 of the 7 samples listed in Table 2 were unsatisfactory since their coatings dissolved in the acid medium in an hour or less. An eighth sample, of which only a few tablets were available, broke up in 15–20 minutes. Work on this problem will be continued, but attention is called to it at this time because of the possibility that it may be tested collaboratively with profit. What is urgently needed is a means of following the preparations through the gastro-intestinal tract of animals by means of X-rays or otherwise to obtain data to be correlated with these *in vitro* studies. Preliminary experiments emphasize the difficulty of this mode of attack and more groundwork must be laid before this phase can be studied collaboratively.

It is respectfully requested that the recommendation\* for collaborative study of these subjects be considered.

## REPORT ON MISCELLANEOUS DRUGS

By C. K. GLYCART (U. S. Food and Drug Administration,  
Chicago, Ill.), *Referee*

Six reports were received from the associate referees this year. No work was done on one topic. Three subjects were recommended to be closed, viz. Iodine Ointment, Mercury Ointment, and Sirup of the Bromides. Four subjects were recommended to be continued, viz. Microchemical Tests for Alkaloids and Synthetics, Magnesium Trisilicate, Emulsions, and Compound Ointment of Benzoic Acid.

*Microchemical Tests.*—The microchemical tests for alkaloids and for synthetics have been combined this year as one topic. Satisfactory tests were developed for physostigmine, dilaudid (dihydromorphinone hydrochloride), sulfapyridine, and sodium sulfapyridine.

\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 55 (1941).

In view of the results obtained by the collaborators, the associate referee recommends that the above-named microchemical tests be adopted as tentative. It is also recommended that the study next year include benzedrine, metrazol, and sulfathiazole. The Referee concurs.

*Iodine Ointment.*—The associate referee has devoted seven years of intensive study to this topic. Early in the work he found that a small quantity of free iodine was absorbed by the ointment base to form organically combined iodine, which increased with the age of the ointment. Uniform results for organically combined iodine were not obtained by the collaborators.

Some years ago a method for the determination of total iodine was developed by the associate referee and was included in the U.S.P. A method for free iodine was adopted tentatively in 1938 with the provision that official status be deferred until methods for free, inorganically, and organically combined iodine were developed.

The work this year consisted of the study of a double titration method for uncombined and inorganically combined iodine. The free iodine was determined by titration with thiosulfate solution and starch indicator; the total iodide was then titrated with silver nitrate solution with para-ethoxychrysoidine indicator.

Two specimen samples were submitted for collaborative study—one a freshly prepared ointment, the other 18 months old. The results are considered satisfactory.

The recommendation by the associate referee that the present method for iodine be modified to conform to the procedure used this year would entail a change from potassium arsenite solution, which was employed in 1937, to the conventional titration with sodium thiosulfate. The reason for the change is that the method for the determination of potassium iodide with the new adsorption indicator is made available.

Since methods are now available for the determination of total iodine, free iodine, and inorganically combined iodine (KI), it is recommended\*—

(1) That the present tentative method for the determination of iodine, *This Journal*, 20, 572 (1937); *Methods of Analysis*, A.O.A.C., 1940, 619, be deleted.

(2) That the method for the determination of iodine employing titration with thiosulfate solution studied this year be substituted and adopted as tentative.

(3) That the method for inorganically combined iodine (KI) be adopted as tentative.

(4) That the subject be closed.

*Magnesium Trisilicate.*—This is a new subject. A limited study of basicity and loss on ignition was made by two collaborators this year.

The associate referee recommends continued study of magnesium trisili-

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\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 54 (1941).

cate to include methods for loss on ignition, basicity, magnesium, silica, free alkali, soluble salts, and adsorption for next year's collaborative work. The recommendation is approved.

*Mercury Ointment.*—In response to the assignment of this topic to consider the possibility of unifying the methods for the mercury ointments, the associate referee submitted a review of work since 1930. This included summaries of methods for the assays of calomel ointment, mild mercurial ointment, and citrine ointment. After a discussion of the application of the thiocyanate and sulfide precipitation methods, he concluded it would be impossible to correlate the methods and recommends that the methods be permitted to prevail in each case.

He also submitted a report for ointment of red mercuric oxide that was assayed by the sulfide precipitation method. The results were 99.98 per cent and 100.04 per cent. He stated that on inquiry a commercially manufactured product was not obtainable.

For this reason and also that the N.F. VII will include an assay if the article is retained, according to information received by him, the associate referee recommends that the subject be closed. The Referee concurs.

*Emulsions.*—No report was received on this topic. It is recommended that the subject be continued.

*Compound Ointment of Benzoic Acid.*—This topic was studied for the first time this year. It involves the quantitative determination of benzoic and salicylic acids in the N.F. article.

The associate referee has made an excellent report of progress, which includes investigation of the accuracy of the bromination of salicylic acid separately and in the presence of benzoic acid.

A simple accurate method for the determination of each acid has been devised, and it will be submitted to collaborators next year.

It is recommended that the subject be continued.

*Sirup of the Bromides.*—Instead of Elixir of Three Bromides, which was originally assigned as a topic by the Committee last year, the associate referee devoted his attention to Sirup of the Bromides, for which no assay is provided in the N.F.

Apparently a commercial product was submitted for collaborative study. The work consisted chiefly of the application of standard methods for the evaluation of total bromine—potassium, sodium, ammonium, calcium, and lithium—in the presence of a large quantity of sugar sirup.

The results are sufficiently close for the bromine content, also for the alkalies, with the exception of sodium, which may be due to incomplete volatilization of the bromine as pointed out by one of the collaborators.

Recently bromides have attained an increased importance under the new Food, Drug, and Cosmetic Act, where the dosage may be unsafe or dangerous to health.

It appears that the work this year has served the purpose so far as Sirup

of the Bromides is concerned. The recommendation of the associate referee that the method be adopted as tentative and that the topic be closed is approved.

*New Topics.*—It is recommended that qualitative tests be studied for ethyl alcohol, isopropyl alcohol, and acetone—preferably by the melting points of the derivatives formed with a reagent such as 3:5 dinitrobenzoic acid.

A rapid method of assay for thyroid in tablets and other unofficial preparations has been used with success for a number of years. The method differs from the U.S.P. assay for thyroid in the substitution of free chlorine for chlorinated soda and the addition of sodium formate instead of boiling off chlorine.

The time required for the assay is reduced to two hours. The end point is sharp and the blank is reduced. The assay was introduced by Morris L. Yakowitz, San Francisco Station.

It is recommended that the method be studied.

## REPORT ON MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS

By GEORGE L. KEENAN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The work on this subject during the past year was confined to testing out significant microchemical reactions for two alkaloids (physostigmine and dilaudid) and two synthetics (sulfapyridine and sodium sulfapyridine). Detailed directions for making the tests on the four substances were prepared and submitted to the collaborators, together with samples Nos. 1, 2, 3, and 4, consisting respectively of physostigmine, dilaudid, sodium sulfapyridine, and sulfapyridine. The controls used, from reputable manufacturers, were considered to be sufficiently pure for these tests.

The directions for making the microchemical tests on these substances and comparison with controls are as follows:

### PHYSOSTIGMINE

*Reagent: Gold bromide in HCl.*—To 1 gram of gold chloride and 1.5 ml. of 40% HBr, add HCl to make 20 ml. (A saturated solution of NaBr may be substituted for the HBr.)

*Preparation of sample.*—Add 1 mg. of the substance to 1 drop of distilled water on a microscopical slide.

*Identification.*—Add a drop of reagent to side of drop containing the substance, apply cover-glass, and examine at a magnification of 100–150. The resulting crystals will consist of brown dendritic aggregates (fern-like) (Fig. 1).

### DILAUDID

*Reagent: Na nitroprusside (solid).*

*Preparation of sample.*—Dissolve a minute quantity of the substance (less than 1 mg.) in 2 drops of water.



FIG 1. -PHYSOSTIGMINE WITH GOLD BROMIDE. ( $\times 300$ )

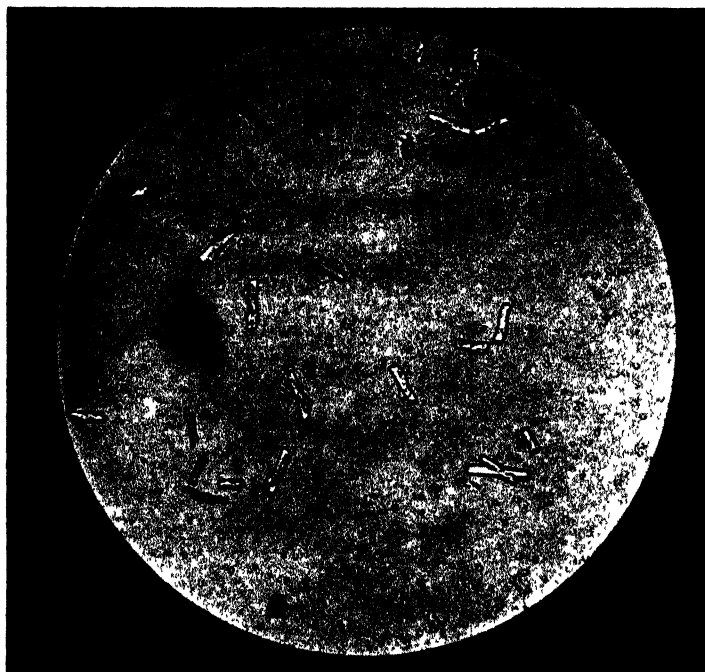


FIG 2. SODIUM SULFAPYRIDINE WITH GOLD CHLORIDE. ( $\times 150$ )



FIG. 3.—DILAUDID WITH SODIUM NITROPRUSSIDE. ( $\times 200$ )

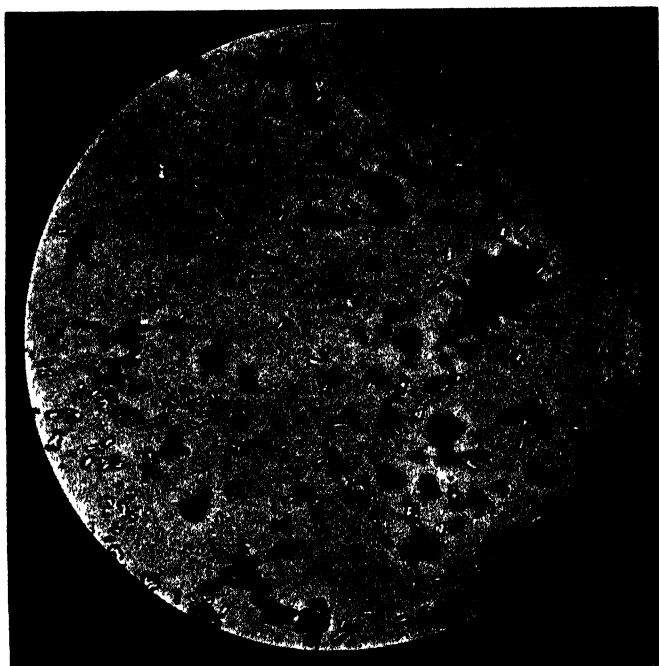


FIG. 4 SULFAPYRIDINE WITH GOLD CHLORIDE. ( $\times 150$ )

**Identification.**—Into the water solution of the substance, drop a minute fragment of Na nitroprusside. The crystalline precipitate immediately forms around the fragment, and upon microscopical examination at a magnification of 100–150 elongated 6-sided prisms will be observed, also occurring in aggregates (Fig. 3).

#### SODIUM SULFAPYRIDINE MONOHYDRATE

**Reagent: Gold chloride solution.**—Dissolve 1 gram of gold chloride in 20 ml. of distilled water.

**Preparation of sample.**—Dissolve 1 mg. of the substance in 2 drops of water to make ca. a 1–100 solution.

**Identification.**—Place a drop of the reagent adjacent to the water solution of the substance and draw it into margin of the test-drop with a clean glass rod. Yellow rods, in x-shaped aggregates, will be observed upon microscopical examination at a magnification of 100–150. After addition of the reagent the drop becomes yellow, gradually darkening at the periphery (Fig. 2). Compare with sulfapyridine.

#### SULFAPYRIDINE

**Reagent: Gold chloride solution.**—Dissolve 1 gram of gold chloride in 20 ml. of distilled water.

**Preparation of sample.**—To 1 mg. of the substance on a microscopical slide, add 1 drop of acetone and 2 drops of distilled water and stir material in with a clean glass rod. (All the material will not dissolve at once in this mixture, but there will be sufficient for the microchemical test (Fig. 4).)

**Identification.**—Place a drop of reagent next to test-drop of substance, drawing it in gently with a clean glass rod, without stirring or covering, and examine microscopically at magnification of 100–150. Yellow rods or blades will be observed, also occurring in x-shaped aggregates. (The crystalline precipitate obtained with sulfapyridine is quite similar in habit to that characteristic of Na sulfapyridine with the same reagent, but the test-drop does not become brown at the periphery in the case of sulfapyridine. It is always observed, however, in testing for Na sulfapyridine monohydrate.)

#### RESULTS AND COMMENTS

The following results were reported by the collaborators, all members of the U. S. Food and Drug Administration, with one exception.

*Chris K. Glycart, Chicago.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

The gold bromide reagent prepared as directed with hydrobromic acid, 40%, produced brown dendritic crystals with physostigmine as described. When a saturated solution of sodium bromide was substituted, the same results were obtained. Instead of mixing these reagents, I also made the test by adding a drop of gold chloride, then a drop of NaBr, and a drop of strong HCl.

The results by the microchemical test for sulfapyridine when glycerol-water solvent was used showed that the latter dissolved the sulfapyridine more slowly than did acetone.

*Samuel Alfend, St. Louis.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

I did not obtain good results at first with a very small crystal of sodium nitroprusside but when I added a crystal weighing approximately 1 mg. and crushed it with a stirring rod, rubbing the preparation two or three times, I obtained at once the characteristic crystals. I suggest that the words "very minute crystal" be eliminated.

Sodium sulfapyridine monohydrate is easily distinguishable from sulfapyridine. When reagent flows into the test solution, a gelatinous yellow precipitate forms at



the edge of the reagent front and darkens to a reddish-brown in a few seconds. Back of it the light yellow crystals form. Some are x-shaped aggregates, but most are not. The sulfapyridine crystals are darker in color than the sodium sulfapyridine monohydrate crystals, and are generally broader.

*Frederick M. Garfield, St. Louis.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

No difficulty was encountered in the identification of the substances.

*Sam D. Fine, St. Louis.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

In the case of dilaudid, crystals did not form immediately with small fragments of sodium nitroprusside. Stirring brought about formation of the crystals. In the case of sodium sulfapyridine, a darkening immediately occurs at the juncture of reagent and water solution of substance with the formation of small, fine, yellow rods. None of the large blades such as occur with sulfapyridine were seen.

*Oliver A. Duff, New Orleans.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

No difficulty was encountered in identifying the compounds.

*E. C. Deal, New Orleans.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

In the case of dilaudid, it was necessary to decrease the size of the sample to approximately 0.2 mg. in order to get good typical crystals. All tests were found to be satisfactory and very distinctive.

*Jonas Carol, Chicago.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

It was found necessary to warm the sulfapyridine and 50% glycerol in order to dissolve the material.

*Irwin S. Shupe, Baltimore.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

In the case of physostigmine, an amorphous precipitate first formed, followed by the dendrites. As a substitute for acetone and water, Shupe suggested the use of 50% glycerol as solvent for the sulfapyridine.

*Morris L. Yakowitz, San Francisco.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

The tests in general were very satisfactory. The test for sulfapyridine requires a little more time, probably due to the slow dissolution of the solid in the glycerol-water mixture.

*Paul S. Jorgensen, San Francisco.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

No difficulty was experienced in identifying the substances.

*Charles C. Fulton, Alcohol Tax Unit, Internal Revenue Service, St. Paul, Minn.*—No. 1, physostigmine; No. 2, dilaudid; No. 3, sodium sulfapyridine; No. 4, sulfapyridine.

I recommend gold bromide in concentrated HCl for dilute solutions of physostigmine but not for concentrated solutions, the crystals being of the characteristic feathered form in the case of the former, while the brown dendritic aggregates are more prevalent in the concentrated solutions. The test for a dilaudid was not considered exceptionally sensitive although the use of a minute quantity of substance is stressed. Sodium sulfapyridine with gold chloride gave a multitude of small crystals with a heavy dark brown precipitate at the edge of the drop. When the substance is dissolved in a drop of water and a drop of dilute acetic acid (20%) is added, a white precipitate is formed, it quickly crystallizes in rosettes of needles, also highly birefringent "worm-like" rods or prisms. It was found to be more convenient to use acetone instead of 50 per cent glycerol for dissolving sulfapyridine.

## SUMMARY AND RECOMMENDATIONS

The collaborators correctly identified the unknown samples submitted and had very little difficulty in applying the tests as described. The few slight variations suggested by some of the collaborators might make the procedure more convenient in some instances. Particular reference is made to the recommendation of Glycart for the substitution of gold chloride solution, sodium bromide solution, and finally strong hydrochloric acid as the reagent for physostigmine. There appears to be no valid objection to this procedure since the final results obtained are as significant as when the reagent described was used.

In view of the results obtained on these microchemical tests for physostigmine, dilaudid, sulfapyridine, and sodium sulfapyridine, it is recommended\* that they be adopted as tentative. It is also recommended that the study next year include benzedrine, metrazol, and sulfothiazole.

## REPORT ON IODINE OINTMENT

By WILLIAM F. REINDOLLAR (State of Maryland Department of Health, Baltimore, Md.), *Associate Referee*

Two samples of iodine ointment, one freshly prepared (A) and the other about 18 months old (B), were submitted to collaborators with the following procedures.

*Iodine*.—Weigh ca. 2 grams of iodine ointment into a 250 ml. iodine flask. Melt on water bath (not above 70°C.), add 30 ml. of  $\text{CHCl}_3$ , mix well, and then add 30 ml. of water. (All of base should be dissolved in the  $\text{CHCl}_3$  before the water is added.) Titrate with 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$ , using starch indicator. Approach the end point dropwise, shaking flask vigorously to make sure that all iodine has been extracted from the  $\text{CHCl}_3$  layer. 1 ml. of 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  = 0.01269 gram of iodine.

*Potassium Iodide*.—Pour the liquids from the iodine determination into a 500 ml. iodine flask, rinsing the former flask with 200 ml. of water, divided into several portions. (It is desirable to maintain this volume within rather narrow limits.) Add 0.5 ml. of 0.2% alcoholic *p*-ethoxychrysoidin indicator and 1–4 drops (to neutralize) of 0.1 *N* NaOH solution. (The aqueous layer should now be clear yellow.) Titrate with 0.1 *N*  $\text{AgNO}_3$ , approaching end point dropwise and rotating flask frequently. (The  $\text{AgNO}_3$  solution causes turbidity due to the formation of colloidal AgI and development of a reddish-brown color similar to that observed in an over-titrated Volhard determination. The end point, which is produced by 1 drop of the volumetric solution, is characterized by flocculation of the colloidal AgI and complete disappearance of the reddish-brown tinge, which leaves an almost clear, pale yellow supernatant liquid.) Quantity (ml.) of 0.1 *N*  $\text{AgNO}_3$  — ml. of 0.1 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  = ml. consumed by the iodide originally present. 1 ml. of 0.1 *N*  $\text{AgNO}_3$  = 0.0166 gram of KI.

The procedure for free iodine is essentially that of the present tentative method, *Methods of Analysis, A.O.A.C.*, 1940, 619, 185, slightly modified to permit the carrying out of both determinations on the same portion of sample. As the method for potassium iodide employs an absorption in-

\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 54 (1941).

indicator with an end point that might be confusing to the inexperienced, the collaborators were requested to make a preliminary titration in order to familiarize themselves with it. The results are presented in Table 1.

TABLE 1.

COLLABORATOR	DATE 1939-1940	SAMPLE A (PREP. NOV. 1939)				SAMPLE B (PREP. JULY 1938)			
		IODINE (AV.)		POTASS. IODIDE (AV.)		IODINE (AV.)		POTASS. IODIDE (AV.)	
Associate Referee	Nov. 9					3.55		4.14	
	10					3.58		4.08	
						3.58		4.06	
						3.52	3.56	4.04	4.08
	14	4.09		4.32					
		4.08	4.09	4.32	4.32				
H. E. Chaney	Nov. 15-	4.14		4.38		3.51		4.17	
	16	4.09		4.35		3.47		(4.39)*	
		4.08		4.36		3.47		4.21	
		4.06	4.09	(4.57)*	4.36	3.53	3.50	4.20	4.19
H. J. Fisher	Dec. 12	4.21		(4.52)*		3.57		4.07	
		4.09		4.01		3.52		4.09	
		4.15	4.15	4.08	4.05	3.58	3.56	4.00	4.05
H. J. Fisher	Dec. 21	4.08		4.24		3.58		4.00	
		4.03	4.06	4.29	4.27		3.58		4.00
L. E. Warren	Jan. 26	3.98		(4.63)*		3.56		4.09	
		4.04		4.29		3.60		4.31	
		3.99		4.25		3.54		4.22	
		4.02	4.01	4.33	4.29		3.57		4.31
Associate Referee	Feb. 12	3.90		4.45					
		3.93				Feb. 15			
		4.00		4.35		3.24		4.43	
		3.97	3.95	4.43	4.41	3.28	3.26	4.49	4.46
E. M. Hoshall	Feb. 24	3.97		(4.91)*		3.50		4.28	
		3.99		4.65		3.51		4.38	
		4.01	3.99	4.68	4.67	3.52	3.51	4.32	4.33
Jonas Carol	Mar. 8	3.99				3.41			
		4.02	4.01			3.43	3.42		
Associate Referee	Mar. 23					3.49		4.17	
						3.45	3.47	4.09	4.13
Jonas Carol	Apr. 10	3.97		4.38		3.45		4.31	
		3.98	3.98	4.34	4.36	3.49	3.47	4.30	4.31

\* Results in parentheses, said by collaborators to be trial determinations, are not included in the averages.

## COMMENTS OF COLLABORATORS

*H. J. Fisher.*—I found the end point with *p*-ethoxychrysoidin quite sharp in a good light. However I had to use more indicator than your directions called for.

*L. E. Warren.*—The high result for potassium iodide in the first assay of Sample A probably is due to overtitation owing to lack of familiarity with the method. The procedure would appear to be satisfactory although it requires judgment in the second titration to ascertain the end point.

*E. M. Hoshall.*—Suggests several minor changes among which was weighing of the sample on a piece of paraffined paper and dropping it into the 500 ml. iodine flask, thus eliminating the use of the 250 ml. flask.

*J. Carol.*—The end points with the indicator ethoxychrysoidin and silver nitrate solution in my first titrations were unsatisfactory, apparently due to excessive alkalinity of the added distilled water. A sharp end point was obtained by first neutralizing the distilled water, and then adding one drop of 0.1 *N* NaOH according to directions in the method.

A review of the collaborative data shows the results for free iodine to be quite satisfactory, but in the case of potassium iodide the figures show marked variation, although the individual collaborators were able to obtain concordant results. In order to obtain an explanation for this discrepancy ointment A was remixed, and a number of samples weighed out immediately after mixing were assayed at approximately 30 day intervals. These data are presented in Table 2.

TABLE 2

DATE 1940	IODINE		POTASSIUM IODIDE	
		AVERAGE		AVERAGE
Apr. 15	3.86	3.85	4.56	4.55
	3.84		4.53	
May 18	3.95	3.96	4.55	4.51
	3.97		4.47	
June 15	3.95	3.95	4.38	4.48
	3.95		4.58	
July 18	3.98	3.99	4.33	4.35
	3.99		4.37	

An examination of all the results seems to indicate that the method measures the potassium iodide present in the portions weighed out, and that these portions are not always representative for this salt. Iodine ointment is a heterogeneous mixture of a glycerol solution of iodine and potassium iodide in a base composed largely of petrolatum, and it is probable that the iodine, which is soluble in both phases, becomes evenly dispersed, while the iodide, which is not soluble, remains in solution in the glycerol.

It is recommended\* that the present method for iodine in iodine ointment be modified to conform to the procedure herein described, and that the method for potassium iodide be adopted as tentative.

\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 54, 93 (1941).

## REPORT ON MAGNESIUM TRISILICATE

By E. K. TUCKER (Alabama Department of Agriculture and Industries, Montgomery, Ala.), *Associate Referee*

The study of magnesium trisilicate during the year consisted mainly of a search of the literature for information concerning its properties, uses, and methods of analysis.

The tests for loss on ignition, basicity, and methylene blue (adsorption) used by Norman Glass,<sup>1</sup> received a limited study in the laboratory by two analysts working independently.

## METHOD

*Loss on Ignition.*—Strongly heat 0.5 gram to constant weight. Results obtained from heating 0.5 gram samples at 650°C. and 750°C. follow:

PERIOD OF IGNITION	LOSS IN WEIGHT AT 650°C.	LOSS IN WEIGHT AT 750°C.
hours	per cent	per cent
1	17.80	22.60
2	18.32	22.90
3	18.70	22.96
4	18.94	23.00
5	19.00	23.00

*Basicity.*—Take 0.2 gram of the sample, transfer to a 100 ml. stoppered bottle, and add 50 ml. of 0.1 *N* HCl. Shake at intervals over a period of exactly 2 hours, and then titrate the excess acid with 0.2 *N* NaOH, using methyl orange as indicator. Express results as mls of 0.1 *N* HCl required to neutralize 1 gram of sample, both "as received" and on the ignited material.

*mls 0.1 N HCl/1 g. sample, "as received"*

Analyst (A): (1) 113.80

(2) 113.30

(3) 113.55

Analyst (B): (1) 113.30

Results on the proposed methylene blue (adsorption) test used by Glass were not satisfactory on the sample used. The method of determination of magnesium and silica used by Glass was not examined in the laboratory.

Appreciation is expressed to J. H. Nicholson and W. J. Marsh of the Alabama Department of Agriculture and Industries for the analytical work reported.

In a review before the mid-year meeting of the American Pharmaceutical Manufacturers' Association, Washington, D. C., December 1938, John F. Ross and Albert Q. Butler presented a most comprehensive study of magnesium trisilicate, including its properties, clinical uses, and methods of analysis. These authors proposed a definition for magnesium trisilicate, powdered, and suggested tests for its identity and purity, including tests for fineness, loss on ignition, assay for magnesium oxide, assay for silica, basicity, adsorptive power, heavy metals, arsenic, soluble salts, sulfate, free alkali, and chloride.

<sup>1</sup> *Quart. J. Pharm. Pharmacol.*, 9, 445 (1936).

It is the opinion of the Associate Referee that the tests mentioned by Glass and by Ross and Butler are worthy of consideration by this Association as subjects for collaborative study. The following remarks refer to the methods of these separate authors:

(1) The method for loss on ignition proposed by Ross and Butler specifies the use of covered crucibles, and contains directions for heating and cooling that are not included in the method proposed by Glass. No temperature limits are given by either of the authors, though Ross and Butler specify the use of a Bunsen burner in the beginning and a Meker burner for the completion of the ignition.

(2) The methods for basicity differ as to concentration of HCl and time allowed for reaction. Ross and Butler proposed a fixed temperature of reaction, used 0.05 *N* HCl, and allowed 4 hours' reaction time. Glass used 0.1 *N* HCl and allowed 2 hours' reaction time. Since this is a determination of a clinical value, the specification of body temperature for the reaction seems logical, while the use of 0.1 *N* HCl would perhaps be more in line with the purpose of the drug, that is, to counteract higher than usual acidity in the stomach. The normality of the stomach of healthy individuals is equivalent to approximately 0.05 *N* HCl.

(3) Ross and Butler propose the use of a covered weighing bottle and the weighing of samples therefrom by difference. This is a refinement necessary because of the nature of the drug.

(4) The assay for magnesium by these authors differs in principle. The one depends upon the titration of  $\text{H}_2\text{SO}_4$  used in a reaction with the sample and the other depends upon the conventional precipitation of magnesium as phosphate from the solution left from the silica determination.

(5) The assay for silica by the separate authors differs in that one uses HCl and the other  $\text{H}_2\text{SO}_4$  for the formation and dehydration of the silicic acid.

(6) Neither of the authors makes claims that his method for methylene blue adsorption should be used as a standard criteria in judging the drug, but since the adsorptive power apparently is a valuable characteristic of the drug, suitable methods should be sought for its estimation.

(7) Tests for free alkali, soluble salts, and sulfates would prove of value in determining the purity of the drug, assuring that the basicity is due to magnesium trisilicate rather than to soluble alkalies, and proving the thoroughness of the washing received during manufacture.

(8) The tests for heavy metals and the tests for arsenic and chlorides are of value because of the possibility of the use of impure ingredients in manufacture.

The Associate Referee recommends\* continued study of magnesium trisilicate, with the possible subjection of methods for loss on ignition, basicity, magnesium, silica, free alkali, soluble salts and adsorption to collaborative study.

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## REPORT ON MERCURY OINTMENTS

By PAUL S. JORGENSEN (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

The subject of mercury in ointments has received attention by this Association at various times over a period of several years. The general principles involved in this determination are few, but the modifications

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\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 55 (1941).

suggested in order to obtain satisfactory results are many. Because of the many modifications in the methods reported in the literature it was recommended that consideration be given to the possibility of unifying the methods with the view to the adoption of a uniform method for ointments of mercury and its compounds, *This Journal*, 23, 58 (1940).

In a preliminary report by Roe, *Ibid.*, 13, 311 (1930), it was indicated that a modified iodometric procedure appeared to give satisfactory results for the assay of calomel ointment and that a modification of the U.S.P. thiocyanate method for stronger mercurial ointment seemed to give satisfactory results when applied to ointment of mercuric oxide. However, in the report by Roe the following year, *Ibid.*, 14, 312 (1931), it was concluded that the thiocyanate method was not satisfactory for this product, but that the modified iodometric method gave reliable results for calomel ointment. This method was accordingly adopted as tentative.

In 1932, Pappe, *Ibid.*, 15, 409 (1932) reported the results obtained by the hydrogen sulfide precipitation method and by the formaldehyde method for the assay of mercuric oxide ointment. It was concluded that the hydrogen sulfide method gave high results and was in general unsatisfactory, and that the results by the formaldehyde method did not show the desired agreement.

Cavett, *Ibid.*, 18, 520 (1935), reported that the ointment base interfered with the determination of mercury in mild mercurial ointment when assayed by the thiocyanate method official in the U.S.P. X. The method was modified in order to remove the base, and this modified method then gave satisfactory results. It was adopted as tentative and after further modification was adopted by U.S.P. XI.

Moraw's report on citrine ointment, *Ibid.*, 21, 579 (1938), indicated that the sulfide precipitation method could be applied to this product with satisfactory results but offered the usual criticism that the time involved in drying and removing precipitated sulfur made this method generally undesirable for routine analysis. Moraw applied the modified thiocyanate method proposed by Wright,<sup>1</sup> to citrine ointment but found that the end point in the titration was indefinite and unsatisfactory. He then applied the acid potassium permanganate treatment as outlined by Hillebrand and Lundell<sup>2</sup> to the filtered nitric acid solution of the mercury salt and concluded that this method gave correct results. His report states further that he tried this method on a sample of mercuric oxide ointment and found it to work satisfactorily, and he was therefore of the opinion that it would be advantageous, from the standpoint of time saved and accuracy, to use this method in preference to the gravimetric sulfide method.

In 1939 Moraw, *This Journal*, 22, 743 (1939), reported the results obtained for citrine ointment on a method essentially the same as that used

<sup>1</sup> *J. Am. Pharm. Assoc.*, 24, 102 (1935).

<sup>2</sup> *Applied Inorganic Analysis* (1929), p. 173.

in his previous report but containing certain refinements that apparently gave more uniform and consistent results. A later report<sup>3</sup> gave the results obtained by the hydrogen sulfide precipitation method on ointment of yellow mercuric oxide. The method as used was essentially the official U.S.P. XI procedure for this product with some modification in the details of manipulation. Moraw concluded that the results obtained were reasonably uniform and apparently satisfactory.

The determination of mercury by the thiocyanate method is generally preferred in those cases where the mercury can be got into nitric or sulfuric acid solution without introduction of chlorine. Mercuric chloride does not react with thiocyanate, and the method therefore fails if hydrochloric acid or chlorides are present. Mercurous salts must be absent because these react with thiocyanate to form metallic mercury and mercuric thiocyanate. However, this method is especially desirable because it can be used when mercury is in the presence of several metals that are more or less difficult to remove in ordinary separations. The sulfide precipitation method is an accurate procedure if carried out properly, but is less desirable than the thiocyanate titration because mercury must be separated from all other metals of the hydrogen sulfide group and also because sulfur may be thrown down and it is sometimes difficult to remove completely.

A general review of the methods for the determination of mercury in ointments as well as other preparations of mercury is given by Garratt.<sup>4</sup> He states that some of the salts of mercury appear to be slightly soluble in fats and difficult to remove from the ointment base with dilute acids, especially when wool fat is present.

In view of the difficulties experienced by the several associate referees of this Association in working out the details of the methods of assay for mercury and its salts in ointments it appears that it will be impossible to correlate the methods for the sake of uniformity except at the expense of simplicity and accuracy, which of course is not desirable. Since accurate methods have been worked out in fine detail for each product it is recommended\* that these methods be permitted to prevail in each particular case.

#### OINTMENT OF RED MERCURIC OXIDE

Ointment of Red Oxide of Mercury is official in National Formulary VI, but no method of assay for the mercuric oxide is included. It was therefore considered advisable to study this ointment and devise a method for its determination.

An ointment was prepared according to the official formula with red mercuric oxide that assayed by the sulfide precipitation method 99.98 and 100.06 per cent mercuric oxide, and by Rupp's formaldehyde method 99.91 per cent mercuric oxide. The prepared ointment was then assayed

<sup>3</sup> Bulletin of Drug Analysis, Vol. 1, p. 41.

<sup>4</sup> Drugs and Galenicals, p. 201 (1937).

\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 55 (1941).



by Rupp's formaldehyde method with the following results: 10.98, 9.54, 9.3, 9.14, 9.87, and 9.92 per cent mercuric oxide. These results were not considered satisfactory and it was thought that before applying other methods to the product it would be desirable to obtain a commercial ointment, inasmuch as it would probably be more uniform. On inquiry it was learned that the ointment is no longer manufactured by the pharmaceutical concerns and it was therefore decided that the product did not merit further attention of the Association at this time, since it was not an article of commerce.

The Associate Referee has been informed that N.F. VII will contain assay methods for the official ointments of mercury. Presumably if ointment of red mercuric oxide is retained as an official product it will have a method of assay. Therefore it is recommended that this subject be closed.

No report on emulsions was given by the associate referee.

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## REPORT ON COMPOUND OINTMENT OF BENZOIC ACID

By WILLIAM F. KUNKE (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Compound Ointment of Benzoic Acid is a new subject this year. It is a National Formulary VI product, and according to the formula it should contain 12 per cent of benzoic acid and 6 per cent of salicylic acid. No assay is given. A partial review of the literature revealed no specific method for the quantitative determination of benzoic and salicylic acids in this product.

Considerable experimental work was done, but no collaborative work was undertaken. Also, no method is proposed, but with the progress made it is hoped that a simple and accurate method can be submitted for collaborative study next year.

Garratt<sup>1</sup> reported a method for the complete analysis of a British Pharmacopoeia Codex 1936 ointment consisting of 5 per cent of benzoic acid and 3 per cent of salicylic acid in a mixture of coconut oil and soft paraffin. He used 2 per cent potassium hydroxide solution to extract the total acids, which besides benzoic and salicylic acids included the liquid acids of coconut oil. In order to avoid so far as possible the saponification or extraction of any constituent of wool fat, which with petrolatum forms the ointment described in the National Formulary VI, it seemed preferable for this investigation to use an aqueous solution of sodium bicarbonate for extracting the benzoic and salicylic acids.

The following procedure, briefly stated, appeared to be worthy of study:

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<sup>1</sup> *Quart. J. Pharm.*, 8, 472 (1935).

Extract the two acids from the ether solution of the sample with an aqueous solution of  $\text{NaHCO}_3$  (ca. 10%). Acidify and extract the acids with chloroform and ether (2+1). Titrate and determine the salicylic acid by bromination and the benzoic acid by difference.

For the titration, 50 per cent alcohol was used as a solvent for the acids. An aliquot of the titration liquid was used for the determination of salicylic acid by bromination, and it was found necessary to remove the alcohol by evaporation because control experiments showed definitely that any alcohol present, even 2 per cent, reacts with bromine. Experiments also showed definitely that there is no loss of benzoic or salicylic acid during evaporation of the titration liquid on a steam bath, and that benzoic acid does not interfere with the determination of salicylic acid by bromination. The benzoic and salicylic acids used in the experimental work complied with all the U.S.P. XI requirements, and without preliminary drying each was found by the U.S.P. XI assay to have a purity of 99.84 per cent.

A batch of the ointment of known composition was prepared and the determinations by weight and titration of the combined acids, benzoic and salicylic, checked well and gave good recovery. However, a highly recommended procedure for bromination of salicylic acid gave a recovery of 95 per cent. The reaction period was 5–10 minutes. This low result for salicylic acid prompted a critical investigation of the conditions necessary for the accurate bromination; namely, excess bromine, acidity, and period of reaction.

With reference to a quantitative determination, the literature is surprisingly meager in giving thorough and critical investigations of the bromination of phenols. The publications of Redman, Weith, and Brock<sup>2</sup> and of Day and Taggart<sup>3</sup> are exceptions. It should be noted that the former study was limited to phenol and that the object of the latter study was primarily to develop "a simple general procedure applicable with the fewest possible individual modifications to the largest number of phenols and aromatic amines." Salicylic acid was included in the total of 28 compounds studied but unfortunately the paper, an abstract of the thesis, gives only the average result of five determinations for salicylic acid, 99.86 per cent.

#### SUMMARY OF BROMINATION EXPERIMENTS

Because of the large number of variables numerous determinations were necessary. As a result of over 100 determinations of salicylic acid with a sample of 0.046 gram of salicylic acid (equivalent to 20 ml. of 0.1 *N* potassium bromide-bromate) and a volume of 100 ml., the optimum conditions were found to be (1) 25 per cent excess bromine, (2) 5 ml. of hydrochloric acid, and (3) 30 minute reaction period. Under these conditions twenty determinations yielded results varying from 99.8 to 100.2 per cent. Small

<sup>2</sup> *J. Ind. Eng. Chem.*, 5, 389 (1913).

<sup>3</sup> *Ibid.*, 20, 646 (1928).

deviations from the conditions given should give equally satisfactory results, but wide deviations will cause poor results. This is shown conclusively by the experimental results obtained with the given conditions constant except for the one deviation noted below:

Deviation	Number of Determinations	Recovery Range per cent
Excess 0.1 <i>N</i> potassium bromide-bromate, 100%	11	100.1-101.0
Reaction period, 2½ hrs.	7	101.5-101.7
Hydrochloric Acid, 20 ml.	4	92.4- 97.3

For each determination precautions necessary for accurate iodometric analysis were taken.

With the progress made it is believed that a simple and accurate method for the determination of both benzoic and salicylic acids can be submitted for collaborative study next year. It is recommended that this subject be further studied.

## REPORT ON SIRUP OF THE BROMIDES

By RUPERT HYATT (U. S. Food and Drug Administration,  
Cincinnati, Ohio), *Associate Referee*

The National Formulary lists Sirup of the Bromides and gives formulas and directions for compounding, but no assay is provided. At the request of Committee B a method of assay was studied. Since in case of a variation in the amount of total bromine it might be necessary to show what particular compound was short or over, the assay was made to include ammonium and the metallic ions.

A large quantity of sugar is present, so some consideration must be given to the method of measuring a portion for analysis. Some experiments were made on a commercial sirup of the bromides to determine total bromine by various methods of measuring the sample.

	grams bromine/100 ml.			
	Volumetric		Gravimetric	
Pipet drained 30 minutes	18.09	18.09	18.19	18.20
Volume calc'd from weight and sp. gr.	18.11	18.12	18.27	18.28
Pipet washed out	18.16	18.16	18.28	18.28

The small difference in the results and the ease of washing out the pipet with a stream of water from the wash bottle made this method appear practical. The gravimetric results were somewhat higher, possibly due to occlusion of sugar in the silver bromide precipitate.

Methods given in *Methods of Analysis*, A.O.A.C., 1940, were used when they were applicable to this mixture.

The methods submitted to collaborators follow:

#### PREPARATION OF DILUTION

Pipet 50 ml. of sirup of the bromides into a 1 liter volumetric flask. Wash out the pipet with a stream of water from a wash bottle, dilute to mark, and mix. Measure out aliquots of this dilution for analysis at the original temperature.

**Total Bromine.**—Transfer 20 ml. of the dilution to a 500 ml. g.s. flask. Add 100 ml. of water, 2 ml. of  $\text{HNO}_3$ , and 25 ml. of 0.1  $N$   $\text{AgNO}_3$ . Titrate the excess of  $\text{AgNO}_3$  with 0.1  $N$   $\text{K-}$  or  $\text{NH}_4\text{SCN}$ , using ferric alum indicator.

1 ml. of 0.1  $N$   $\text{AgNO}_3$  = 0.007992 gram of bromine.

**Ammonium Bromide.**—Introduce a 100 ml. aliquot of the dilution into a Kjeldahl flask, add 100 ml. of water, a small piece of paraffin, and an excess of 10%  $\text{NaOH}$  solution. Distil the ammonia into an excess of standard acid, 40 ml. of 0.1  $N$  usually being sufficient. Titrate the excess of acid with 0.1  $N$   $\text{NaOH}$ , using methyl red indicator.

1 ml. of 0.1  $N$  acid = 0.009796 gram of  $\text{NH}_4\text{Br}$ .

**Calcium.**—Pipet a 100 ml. aliquot of the dilution into a casserole. Evaporate and ignite at dull red until the organic matter is thoroughly charred. Add 5 ml. of 10%  $\text{HCl}$  to dissolve calcium salts, filter, and wash well with hot water. Return filter and unoxidized carbon to casserole and ignite at moderate temperature until the residue is white. Treat the residue with 5 ml. of 10%  $\text{HCl}$ , filter, and wash with hot water, combining the filtrates.

Also determine calcium as directed in *Methods of Analysis*, A.O.A.C., 1940, under XII, 10, and reserve the filtrate for the determination of Na, K, and Li. If 0.1  $N$   $\text{KMnO}_4$  is used, 1 ml. = 0.0100 gram of  $\text{CaBr}_2$ .

**Sodium, Potassium, and Lithium.**—Dilute the filtrate and washings from the calcium determination to 200 ml. and mix. Evaporate a 100 ml. aliquot to dryness and drive off all  $\text{NH}_4$  salts by heating to faint redness in a platinum dish. Treat the residue with a little water, filter into a small platinum dish, add a few ml. of  $\text{HCl}$ , and evaporate to dryness.

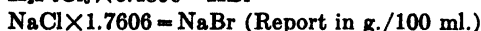
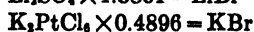
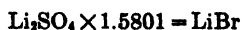
Complete the conversion of the alkali bromides to the chlorides by one of the following procedures:

(1) Dissolve the residue in a little water, add 5 ml. of  $\text{HCl}$ , and evaporate to dryness. Repeat the addition and evaporation of water and  $\text{HCl}$  three additional times, dry thoroughly in the oven, and heat carefully to avoid decrepitation of  $\text{NaCl}$ . Cool, and weigh. Dissolve in water, add 5 ml. of  $\text{HCl}$ , evaporate, heat, and weigh as before. If dish and contents are not to constant weight (within 0.5 mg.), continue the evaporation with  $\text{HCl}$  until constant weight is obtained. Proceed as directed under XXXVII, 65, beginning "Dissolve mixed chlorides in hot  $\text{H}_2\text{O}$ , filter, and wash."

(2) Prepare the alkali sulfates by evaporating with an excess of  $\text{H}_2\text{SO}_4$  and expelling the excess by careful ignition. Repeat the evaporation with  $\text{H}_2\text{SO}_4$ , cool, and dissolve the mixed sulfates in water. Add 1 ml. of  $\text{HCl}$ , heat to boiling, and precipitate the sulfates with a small excess of 10%  $\text{BaCl}_2$  solution. Digest, filter, wash, and precipitate the excess barium in the filtrate with  $(\text{NH}_4)_2\text{CO}_3$  and  $\text{NH}_4\text{OH}$ . Filter off the  $\text{BaCO}_3$  and wash the precipitate with water containing a small quantity of  $(\text{NH}_4)_2\text{CO}_3$ . Acidify the filtrate with  $\text{HCl}$ , evaporate to dryness, and drive off all  $\text{NH}_4$  salts by heating to faint redness in a platinum dish. Proceed as directed under XXXVII, 65, beginning "Treat residue with a little  $\text{H}_2\text{O}$ , filter into a small Pt dish, add a few drops of  $\text{HCl}$  (1+1), and evaporate to dryness."

To reduce the amount of platinum solution used, the solution of  $\text{KCl}$  and  $\text{NaCl}$

can be made to 50 ml. and a 25 ml. aliquot taken. The platinum filtrates and residues can be reserved for recovery.



*Results of collaborators (g./100 ml.)*

<i>Name</i>	<i>Total bromine</i>	<i>NH<sub>4</sub>Br</i>	<i>CaBr<sub>2</sub></i>	<i>LiBr</i>	<i>KBr</i>	<i>NaBr</i>
F. K. Killingsworth	18.12	5.10	2.26	0.80	7.96	9.34
	18.12	5.09	2.26	0.80	8.15	9.36
Iman Schurman	18.05	5.13	2.22	0.81	8.17	8.74
	18.13	5.11	2.24	0.96	8.27	9.11
M. M. Spruiell	18.22	5.07		0.75	7.97	8.88
	18.18	5.08	2.09	0.69	7.90	8.82
	18.17					
L. H. Welsh	18.25	5.09	2.22	0.88	8.36	10.62
	18.27	5.10	2.21		8.35	10.96
Jonas Carol	17.91	4.68	2.27	0.75	8.15	10.21
	17.91	4.81	2.31	0.77	8.27	10.09
Rupert Hyatt	18.16	5.08	2.21	0.79	8.08	9.02
	18.16	5.10	2.25	0.86	7.97	9.22

#### COMMENTS BY COLLABORATORS

*L. H. Welsh.*—The procedure was followed as described, except that inadvertently the entire filtrate from the calcium determination was used for the determination of the alkali metals. It may be preferable to use a volumetric flask in measuring the 50 ml. of sirup. Also, the end points observed in the Volhard titrations were not distinct, but were rendered sharp by filtering off the silver halide and titrating an aliquot of the filtrate.

*F. K. Killingsworth.*—I do not think that washing out the pipet is an accurate method of measuring the sample. I suggest that a 50 ml. volumetric flask be filled to the mark and then the contents washed into a liter flask. In this way exactly 50 ml. will have been taken. Washing out a pipet will give slightly higher results as pipets are usually calibrated to deliver the stated quantity.

The quantity of  $\text{Li}_2\text{SO}_4$  is too small for accurate determination. I suggest a larger aliquot. The temperature for heating  $\text{Li}_2\text{SO}_4$  should be specified. Dull redness is recommended by Scott's Standard Methods of Chemical Analysis, Vol. 2, p. 889.

*Jonas Carol.*—The ammonia distillation foamed so badly that it was necessary to use a very small flame. The sodium results are higher than the N. F. formula specifies. That could be caused by incomplete volatilization of the Br while heating the K, Na, and Li residue with HCl.

#### CONCLUSIONS

The results obtained by the analysts are considered to be in good agreement. This is particularly true of total bromine, ammonium bromide, and calcium bromide, as well-known methods were used for their deter-

mination. Smaller quantities of the alkali metals are determined but they are sufficient for the official methods given under water analysis.

Official methods are used, so that only the preparation of the sample for analysis appears to deserve study. In case of shortage of ingredients the washing out of the pipet, which gives a small plus error, would benefit the manufacturer. The error involved is in the range of 0.1–0.2 per cent. If a greater accuracy is desired or if an excess is found, the concentration of the dilution may be determined by weight and specific gravity.

It is recommended\* that the procedure be adopted as a tentative method and study discontinued.

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The paper entitled "Assay of Oil of Peppermint, Oil of Rosemary, and Oil of Sandalwood," presented by D. C. Grove, was published in *This Journal*, 24, 465 (1941). For the paper entitled "Modification of the Assay for Mercuric Nitrate Ointment," presented by R. K. Snyder, see p. 927.

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\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 55 (1941).

# THIRD DAY

## WEDNESDAY—MORNING SESSION

### REPORT ON FEEDING STUFFS

By L. S. WALKER (Agricultural Experiment Station,  
Burlington, Vt.), *Referee*

*Fat in Dry and Canned Dog Feeds.*—It is apparent that there is considerable discrepancy between the official ether-extraction and acid-hydrolysis methods. Since many dog feeds are cooked, the manufacturers claim that all the fat is not obtained by the ether-extraction method and that for the materials classed as cooked cereal food the A.O.A.C. acid hydrolysis method should be used. It is recommended that this method, *Methods of Analysis, A.O.A.C.*, 1940, 213, 11, be used on cooked animal feeds containing cereals.

*Fat in Feeding Stuffs.*—Many of the control and commercial laboratories do not follow strictly the A.O.A.C. method for the determination of crude fat. The official method specifies a 16 hour extraction, whereas many chemists use the new Goldfisch method, which lessens the time of extraction to 4 hours. In *This Journal*, 18, 351 (1935), it was reported that five commercial laboratories had purchased the Goldfisch extraction apparatus and had approved the short-time method and its results. These laboratories reported that there is no question but that this apparatus will make an ether extract as accurately as the 16 hour official method. Since then many manufacturers and control laboratories are using this piece of apparatus to take the place of the 16 hour digestion. In 37 states the A.O.A.C. methods are specified in the feeding stuffs laws. Therefore if any of these states were to prosecute a manufacturer in the case of a low fat product, it would be necessary to use the 16 hour extraction. It would seem that this question is of sufficient importance to warrant some consideration. It is therefore recommended that an associate referee study this subject.

*Acidity in Feeds.*—It has been suggested that the tentative method for the determination of water-soluble acidity under Grain and Stock Feeds, *Methods of Analysis, A.O.A.C.*, 1940, 363, 38, is obsolete and that the method developed by Lawrence Zeleny for fat acidity in grain be substituted. Before this action can be taken, it will be necessary to study the Zeleny method collaboratively. It is therefore recommended that an associate referee be appointed for that purpose.

*Filtration Aid for Crude Fiber Determination.*—A paper by A. M. Neubert *et al.*, Division of Chemistry, Pullman, Wash., on "Potassium Sulfate, a Filtration Aid for the Crude Fiber Determination," shows that this product is of distinct value in the filtration of samples where filtration

ordinarily is slow. As this subject seems to warrant collaborative study and the appointment of an associate referee, it is suggested that such action be taken.

*Grit in Poultry and Similar Feeds.*—It was recommended at the last meeting that the method for the determination of grit in poultry and similar feeds, *Methods of Analysis, A.O.A.C.*, 1940, 365, 46, and bone in meat scraps and tankage, *Ibid.*, 47, be made official (first action).

It has been suggested by W. L. Hunter, Feed Laboratory, Sacramento, Calif., that some amendments are desirable before final action is taken on these methods. The suggestions made are—

(1) That for the sake of economy and safety, the use of  $\text{CHCl}_3$ , which is required by these methods, be made optional with  $\text{CCl}_4$ .

(2) That in the method for the determination of grit in poultry and similar feeds, bone and salt be included in the result; that salt be removed by washing with water; and that an easy distinction of bone and minerals is made by heating the sample until the bone chars.

(3) That a statement be made in the method for grit that the method is not applicable to pelleted feeds or feeds containing appreciable amounts of molasses, unless they are first disintegrated by thorough washing in cold water and dried with alcohol or ether.

It is recommended that an explanatory note be added to paragraph 46, p. 365, as follows: "If sample contains salt, wash grit with water; if molasses, remove from original material with water and dry with alcohol and ether. Identify bone in grit by charring.

*Salt in Feeds.*—At the last meeting it was recommended that the paper presented on the determination of chlorine in feeding stuffs, *This Journal*, 23, 425 (1940), be studied by the Referee and if deemed advisable that collaborative work be undertaken. Several samples were tested in this laboratory, and one was sent to G. E. Grattan, Ottawa, Canada. Close agreements were obtained in both laboratories. It would seem that the method is accurate and rapid. Grattan reports that it is giving very satisfactory results and warrants the approval of the Association. It is therefore recommended that an associate referee be appointed and the method submitted to collaborative study.

*Castor Seed.*—About two years ago castor bean cake or hulls were found in feeds on the West Coast. Cottonseed meal denatured with 10 per cent castor bean meal that had been shipped from Japan as a fertilizer was not properly labeled and so found its way into feeding stuffs. Since then none has been found. It is interesting to note that castor beans lose their poisonous principle when subjected to heat during the extraction of the oil. During the past year there has been no indication of the use of castor bean cake in feeding stuffs. It is therefore recommended that this subject be discontinued.

*Urea and Inorganic Nitrogen Salts.*—Urea has been used in experiments and research for ruminant feeding, and there are indications that this



product can be used successfully and economically as a partial protein substitute. Therefore, methods should be studied for its determination. It is recommended that the method submitted by Griem, *This Journal* 24, 79 (1941), be adopted as tentative and that an associate referee be appointed to study this subject.

*Microscopical Analysis.*—Most state feeding stuffs laws require a statement of ingredients on the tags. In order to determine these ingredients microscopical analyses are required. Procedures for the examination and identification of a few food products by means of the microscope are mentioned in *Methods of Analysis*. Feeding stuffs are not mentioned. The Referee desires to know the policy of the Association on this subject. It has been suggested that a committee be appointed to study this question.

*Manganese.*—It is recommended that the method for the determination of manganese (p. 369, 59, 60) be revised as suggested by the associate referee.

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No report on sampling was given by the associate referee.

## REPORT ON ASH

### EFFECT OF ASHING TEMPERATURES ON PERCENTAGE OF MINERAL ELEMENTS RETAINED

By J. L. ST. JOHN (Division of Chemistry, Agricultural  
Experiment Station, Pullman, Wash.), *Associate Referee*

The temperature, time of ashing, and other conditions are definitely specified in the method for the determination of ash proposed in last year's report, *This Journal*, 23, 620 (1940). This method was adopted as official by the A.O.A.C. and incorporated into the 1940 edition of *Methods of Analysis*, A.O.A.C., p. 354.

Fundamentally, the amount of ash in a sample is presumed to have a direct relation to the quantity of mineral matter in the sample ashed. The available literature, however, failed to show that the ash obtained by methods described for the ashing of feeds and other plant material is related to the combined mineral elements in the sample. The results of a study made in the Referee's laboratory by St. John and Midgley (see p. 932) indicate that the percentages of ash are a measure of the relative quantity of certain elements in plant material, while in other cases this relation is not apparent. It is further shown that the temperature of ashing influences the quantity of certain elements that are retained in the ash. On the basis of this information the Associate Referee submitted two samples of feeds practically identical to those used during the course of the studies reported in 1939 and 1940 to the collaborators that had generously assisted in the work during the two previous years, in order to obtain more complete information regarding the relation between the min-

TABLE 1.—*Collaborative results (per cent)*

COLLABORATOR	POULTRY FEED				DAIRY FEED			
	HClO <sub>4</sub>	500°	600°	700°	HClO <sub>4</sub>	500°	600°	700°
<i>Calcium</i>								
Allen		.97	.95	.98		.22	.22	.22
O'Meara-Nasif	.98	.99	.98	.99	.26	.25	.26	.25
Flack-Hasbrouck		1.02	1.00	.98		.25	.27	.26
McClure	.94	.93	.97	.94	.24	.23	.23	.23
Bradford-Matheson	.98	.98	.98	.99	.24	.24	.24	.25
Randall	.88	.99	.98	.97	.20	.27	.26	.25
Midgley-St. John	.99	.98	.99	.98	.24	.24	.24	.25
Hanke	.99	.97	.96	.97	.25	.24	.28	.27
AVERAGE	.961	.980	.977	.975	.235	.243	.245	.244
<i>Phosphorus</i>								
Allen		.88	.90	.90		.47	.47	.46
O'Meara-Nasif	.89	.75	.73	.75	.46	.34	.34	.32
Flack-Hasbrouck		.88	.90	.90		.44	.46	.47
McClure	.89	.85	.89	.87	.46	.43	.44	.44
Bradford-Matheson	.88	.87	.87	.87	.45	.44	.44	.41
Randall	.90	.84	.79	.83	.47	.42	.43	.43
Midgley-St. John	.98	.92	.93	.92	.52	.51	.50	.50
Hanke	.89	.89	.88	.86	.46	.43	.42	.38
Elliott		.71	.91	.89		.51	.53	.51
AVERAGE	.904	.843	.867	.865	.469	.443	.447	.434
<i>Sulfur</i>								
Bicknell		.052	.042	.045		.026	.021	.026
Christie		.056	.046	.026		.030	.018	.012
Swift-Curtis	.166	.057	.043	.040	.106	.028	.021	.021
Blomquist-Ladd	.173	.077	.060	.008 <sup>1</sup>	.105	.041	.015	.007 <sup>1</sup>
Hopper-Majors	.167	.066	.051		.110	.040	.034	
Laing-Vollertsen	.160	.043	.044	.045	.110	.023	.021	.025
Midgley-St. John	.180	.047	.042	.044	.140	.030	.024	.034
AVERAGE	.169	.056	.046	.034	.114	.031	.022	.020
<i>Chlorine</i>								
	<i>Fusion</i>				<i>Fusion</i>			
Adams	.96	.89	.79	.60	.82	.64	.55	.32
Hanna-Frary	.97	.86	.86	.64	.83	.63	.58	.36
Potvin-Marshall	.88	.87	.87	.82	.66	.65	.64	.53
Spears-Terrell	.99	.78	.81	.50	.80	.58	.55	.25
Crowley-Kuzmeski	1.08	.83	.92	.68	.95	.59	.67	.42
Struve	.97	.87	.82	.69	.81	.65	.57	.46
Willis	.94	.85	.72	.51	.81	.60	.43	.13
Zeigler	.93	.84	.83	.71	.82	.66	.62	.37
Walls-Bopst	.93	.84	.84	.55	.86	.61	.57	.24
Midgley-St. John	1.00	.88	.88	.78	.84	.66	.64	.51
AVERAGE	.965	.851	.834	.648	.820	.626	.582	.359

TABLE 1.—Continued

COLLABORATOR	POULTRY FEED				DAIRY FEED				
	HClO <sub>4</sub>	500°	600°	700°	HClO <sub>4</sub>	500°	600°	700°	
		<i>Potassium</i>							
Kaufmann		.77	.74	.52	.72	.65	.64	.43	
Ogier-Fraps		.81	.77	.68		.66	.62	.54	
Hand		.78	.74	.56		.68	.61	.45	
Frederick-Johnson		.62	.62	.61		.65	.55	.46	
Brew-Kishlar	.79	.79	.74	.46	.73	.68	.55	.23	
Morris		.93	.91	.73		.78	.76	.52	
Midgley-St. John	.77	.66	.69	.55	.65	.60	.52	.47	
AVERAGE		.780	.766	.744	.587	.700	.671	.607	.443

eral elements contained in the ash and the total amount present in feeds. The St. John-Midgley paper includes numerous references to the literature.

Another problem, which the Associate Referee hopes to study further, involves the significance of the phrase, "free from carbon," which was also briefly discussed in last year's report. It may not be necessary to study this phase of the problem collaboratively.

One group of collaborators was asked to determine calcium and phosphorus in ash prepared at different temperatures and also the total calcium and phosphorus in the feed by specified methods. Similarly, other groups were asked to determine sulfur, chlorine, and potassium, respectively.

For determining the total amount of the different elements in the feed perchloric acid was used as an oxidizing reagent in all cases except in the determination of chlorine, where the sodium carbonate fusion method was used.

The collaborators were also requested to ash specified quantities of two feeds at 500°, 600°, and 700°C., by the new official method to determine the different elements on the ash thus prepared by the official A.O.A.C. methods or by any slight modification of these methods essential in adapting them to use in this connection. For example, potassium in plant material requires that the sample be ashed after it has been moistened with sulfuric acid. The collaborators were asked to omit the use of sulfuric acid in the procedure.

The average results of the collaborators are presented in Table 1. All results are expressed as per cent of the element.

From the average results shown in Table 1 the percentages of the different elements recovered in the ash of the feed prepared at the three different temperatures were calculated. They are presented as ratios in Table 2, and the following discussion is based on both the data in Table 1 and the calculations in Table 2.

TABLE 2.—*Ratio of elements in ash to total in feed.*

ELEMENT	POULTRY FEED			DAIRY FEED		
	500°	600°	700°	500°	600°	700°
Calcium	1.020	1.017	1.015	1.034	1.043	1.038
Phosphorus	.932	.959	.957	.944	.953	.925
Sulfur	.331	.272	.201	.272	.193	.175
Chlorine	.882	.865	.672	.977	.908	.560
Potassium	.982	.954	.753	.959	.867	.633

The data in Table 1 indicate no loss of calcium when the samples were ashed at any of the three temperatures. The percentages are essentially the same as those obtained by the different collaborators when the samples were digested with perchloric acid and calcium was determined in these solutions. In both feeds there was complete retention of the calcium in the ash under all conditions. On the average the percentage of calcium is slightly higher than it is when perchloric acid digestion is used, although the difference may not be significant. The trend of the data for phosphorus is in the opposite direction and it does indicate some loss of phosphorus during the ashing procedure. The temperature of ashing did not affect the loss of phosphorus. With each feed the loss is essentially the same at all three temperatures.

The data for sulfur show a marked loss at all ashing temperatures, although differences between temperatures are not marked. In general, the perchloric digestion method gave greater uniformity of results among the different collaborators than did the ashing method. The percentage of sulfur obtained by the collaborators varies rather widely. In the poultry feed the maximum recovery in ash is about one-third of the total quantity of sulfur in the feed. Recovery is less at the higher temperatures and sulfur in the ash at 700° is less than one-fourth of the total present in this feed. In the dairy feed about one-fourth of the sulfur was recovered when the ash was determined at 500° while only one-fifth of the sulfur was recovered when temperatures of 600° and 700°C. were used. A difference between the two feeds is evident.

The loss of chlorine during the ashing of the samples was much less than was the loss of sulfur, and it was progressively greater as the temperature of ashing was increased. The loss of chlorine in the two feeds varied at different ashing temperatures, but the type of variation was somewhat different from that of sulfur or potassium. At 500°C. recovery of chlorine in the dairy feed was nearly complete, while the poultry feed lost about 12 per cent of its chlorine. At 600°C. the two feeds varied in the opposite direction, the poultry feed showing the greater loss. At 700°C. the dairy feed had lost materially more chlorine than had the poultry feed, the poultry feed had lost practically one-third of its chlorine, and the dairy feed had lost nearly one-half of the chlorine present in the feed.

Somewhat more surprising than the data for sulfur and chlorine are those obtained for potassium. While only two collaborators presented complete results for potassium by the perchloric acid digestion, the results seem quite definite in showing that there was some loss of potassium during ashing and that the loss was greater in ash obtained at 700°C. The two feeds vary in the loss of potassium at these higher temperatures, the dairy feed showing the greater loss. Ashing at 700°C. caused an average loss of 25 per cent of potassium from the poultry feed, while the dairy feed lost 37. per cent. At 600°C. nearly 15 per cent of the potassium was lost from the dairy feed.

TABLE 3.—*Ratio of percentage of element to percentage of ash*

ELEMENT	POULTRY FEED			DAIRY FEED		
	500°	600°	700°	500°	600°	700°
Calcium	.137	.143	.153	.040	.041	.045
Phosphorus	.118	.127	.135	.072	.076	.079
Sulfur	.008	.007	.005	.005	.004	.004
Chlorine	.119	.122	.101	.102	.098	.065
Potassium	.107	.109	.092	.109	.103	.081

A further comparison is possible from the data presented in Table 3, which shows the ratio of the percentage of each element, determined upon the ash at different temperatures, in its relation to the average percentage of ash in the two feeds at the different temperatures. Previous reports show that the percentage of ash decreases as the temperature increases; therefore, since the quantity of calcium recovered is essentially the same for all conditions (perchloric digestion and the three temperatures of ashing), the ratio element/ash will increase with increasing ashing temperature (Table 3). With phosphorus there is some decrease in the relative amount recovered in the ash (Table 2), but this decrease is not so rapid as is the decrease in the percentage of ash itself in these feeds determined at these temperatures, as shown by the fact that the ratio of element phosphorus/ash given in Table 3 increases with increase in temperature, as in the case of calcium.

With sulfur, chlorine, and potassium, however, the progression of the ratios shown in Table 3 is, in general, reversed. With both feeds, the differences between the ratios at 600° and at 700°C. are materially larger than are the differences between the ratios at 500° and at 600°C., indicating in another way the desirability of ashing at a lower temperature, so far as these elements are concerned, in order to obtain the closest possible correlation between the amount of the elements in the feeds and the percentage of ash. In Table 3 the ratio is rather consistently less for the dairy feed.

## DISCUSSION

In the utilization of these data as an aid in selecting an ashing temperature, four considerations might be emphasized: (a) the percentage of recovery of the different elements, as indicated in Table 2; (b) the ratio of the percentage of the element in the feed to the percentage of ash; (c) the consistency and reproducibility of actual ashing results at the different temperatures; and (d) the extent to which the samples are "free from carbon" at the different ashing temperatures. This report and the two previous reports deal with the first three of these considerations, so far as this may be shown by the study of the five elements selected for this purpose and presented in this report. It is hoped that further evidence regarding the carbon remaining at different ashing temperatures will be available during the next year.

Since, as shown in Table 3, the ratio of element to ash for calcium and phosphorus increases with increase in temperature of ashing and decreases for the remaining three elements, and in view of the relative recovery of the different elements (Table 2), the analyst must select the temperature giving the closest correlation between ash and mineral matter in the original feed, as far as this is shown by the five elements involved in this study. By making a general average of the ratios at each temperature, as shown in Table 3, a more definite indication regarding the desirable temperature to use is obtained, even though the direction of the progression of the ratio is in one direction with calcium and phosphorus and in the opposite direction with sulfur, chlorine, and potassium. Such an average indicates comparatively little difference at the three temperatures with the poultry feed, while with the dairy feed the average ratio is materially less at 700°, and the average ratios at 500° and 600° are nearly the same. Further, if an over-all average of recovery is made of the different elements at the different temperatures (Table 2) there is shown a greater difference in the percentage of recovery between 600° and 700°C. than between 500° and 600°C. Thus, when the five elements are considered, more nearly the same total recovery of these elements is obtained at the temperatures 500° and 600° than at 700°C.

Based on previous reports, consideration (c) indicates that the consistency of results among the different collaborators is practically the same at 600° as at 650°C., which is the temperature recommended in the present official method. Therefore, in the light of considerations (a), (b), and (c) it is believed that a temperature of 600° could well be used for the ashing of samples of this kind. Whether or not it would be advisable to reduce the ashing temperature to 550°C., which is used in ashing flour, or perhaps to 500°, should depend partly on the added information that it is hoped will be available regarding (d) and the relation of this information to (c). Since lower temperatures give more complete recovery of some mineral elements, this latter lower temperature would seem more desirable

from that standpoint (a). It is hoped that a final recommendation can be made during the coming year.

It would seem that the results presented in this report may be of further significance. Together with information recorded by St. John and Midgley these data indicate the desirability of further consideration of the methods of determining mineral elements in plant material of various types, as illustrated more specifically by the paper on the potassium method. The data in these three papers point toward a materially higher recovery of certain mineral elements when the plant material is brought into solution by perchloric acid digestion than when the ashing method is used. An incidental advantage of using a temperature of 600° or 500°C. is the fact that it appears that ashing at these temperatures may be done in Pyrex beakers. Some observations in this laboratory indicate that it is doubtful whether a temperature of 650°C. may be safely used with Pyrex beakers. The use of beakers in ashing at 550° or 600°C. in preparing samples for the determination of mineral elements in a feed or plant sample has an advantage of saving a transfer of material from ashing crucible to beaker if the analyst should prefer to use the present A.O.A.C. ashing procedures rather than the perchloric acid digestion or other similar methods. The loss of potassium at 600° and 700°C., as shown in this report, may be of significance in controlling the ashing temperature in the A.O.A.C. method for the determination of potassium in plant material, to which further consideration should be given. It is also observed that there is better agreement among collaborators when the perchloric acid digestion is used than was obtained by determining the elements on the ash. A further interesting observation results from the fact that there is no loss of calcium during ashing at any temperature, which supports the argument that there is no mechanical loss of ash resulting from placing the feed sample into the muffle, which has already been heated to the temperature to be used in making the ash determination.

#### SUMMARY

The relation of the percentage of ash determined in feeds to the total amount of mineral matter present and the effect of ashing temperature on this relation are shown. The recovery of calcium, phosphorus, and potassium is above that of chlorine and sulfur; two-thirds or more of the sulfur is lost during ashing. All the calcium appears to be retained in the ash. The temperature of ashing has a marked influence on the loss of potassium, sulfur, and chlorine. Ashing at 700°C. causes a relatively greater loss than ashing at 500° or 600°C., indicating the desirability of a lower ashing temperature. The effect of the ashing temperature on the loss of elements, particularly potassium, indicates a need for a further study of methods for the determination of potassium in plant material, with a possible utilization of perchloric acid digestion rather than ashing in preparing such samples for analysis.

## REPORT ON MINERAL MIXED FEEDS

By ALFRED T. PERKINS (Kansas Agricultural Experiment Station, Manhattan, Kan.), *Associate Referee*

During the past year there has been some criticism of the official method for calcium in mineral mixed feeds, *This Journal*, 23, 637 (1940); *Methods of Analysis*, A.O.A.C., 1940, 365, 48, and for the tentative method (Elmslie-Caldwell) for iodine, *This Journal*, 21, 597 (1938). The criticism of the calcium method is that in some mineral mixes containing calcium monophosphate, low calcium results are obtained. This criticism has been checked in the Associate Referee's laboratory. The objection to the iodine method is that in mineral mixes low in organic matter, inaccurate results are obtained. The criticism of the iodine method was published by Johnson and Frederick, *Ibid.*, 23, 688 (1940). The Associate Referee has not completed his investigations of this criticism.

## RECOMMENDATIONS

It is recommended—

- (1) That studies of the official method for calcium be continued.
- (2) That the iodine method proposed by Frederick and Johnson be studied collaboratively in comparison with the Elmslie-Caldwell method.

## REPORT ON LACTOSE IN MIXED FEED

By D. A. MAGRAW (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Collaborative work for 1939, *This Journal*, 23, 640 (1940), showed that the use of alcohol in one step of the procedure was necessary to eliminate interference from peanut meal and low grade tankage. It also was concluded from the evidence presented that the alcohol eliminated the necessity of using a feed blank. This work also suggested a procedure for determining the correction factor for the reducing value of the animal diastase, and indicated a wide variation in fermentation loss when the small Fleischmann yeast cakes were used.

It was recommended that consideration be given to determining a satisfactory fermentation factor on Fleischmann small yeast cakes and whether baker's yeast would be more satisfactory.

Before any definite plans were made for this year's work, the Associate Referee had occasion to work on the determination of dry milk solids not over 1.5 per cent fat in bread, which involves practically the same procedure as that for lactose in mixed feed. This work clearly indicated that if Fleischmann's baker's yeast was washed four or five times with water, the fermentation loss of lactose was much more uniform, and was materially reduced. However, time did not permit an investigation as to what effect the washing might have on the small yeast cakes.



Owing to this work on breads, all the yeast used in this study was Fleischmann's baker's yeast, which had been washed as follows: 25 grams was washed 5 times with 3-4 times its volume of water, and centrifuged each time. The last washings were clear. Twenty-four hours before being used a suspension of the washed yeast was made up to 100 ml. with distilled water and kept at 0°-4°C.

When the washed yeast was used, the lactose fermentation loss was only 2-3 per cent, thus making the fermentation factor 97 per cent, while the unwashed yeast gave variations of 4-15 per cent.

TABLE 1.—*Results on flour middlings*

SAMPLE NO.	LACTOSE	LACTOSE AS REPORTED BY W. B. GRIEM
	per cent	per cent
641	1.87	2.32
619	1.78	2.28-1.51
1	.48	
2	.23	
3	Blank	
4	.53	
5	.27	
13518	.58	
13519	.14	
13520	.48	
13521	.26	
13522	.37	
13621	.29	
13622	.38	
13623	.62	
13624	.43	

In trying to clear up another difficulty that had been encountered, as reported in the 1939 collaborative work, the animal diastase was involved. The Associate Referee again contacted Armour & Company's Pharmaceutical Department (seemingly the only manufacturer of this diastase). The animal diastase that gave satisfactory results was a special product carrying the specifications of 1-40 diastase and 1-80 trypsin. In order to make sure that the same material would be supplied on reordering, the manufacturer specified the batch as No. 102374.

Another difficulty, encountered in the form of an interference, prevented any collaborative work during this year's study. It was felt that this interference should be definitely worked out and settled before any further work was done.

W. B. Griem of Madison, Wisconsin, called attention to the fact that when certain samples of flour middlings were carried through the procedure, the results indicated variable amounts of a reducing substance which, when calculated as lactose, gave amounts up to 2.32 per cent. When this was called to the Associate Referee's attention a number of

flour middlings samples were carried through the procedure. A few of the results on these samples are shown in Table 1.

An effort was then made to determine whether this reducing substance was a sugar or something else. After several attempts to separate or identify the substance as a sugar had been unsuccessful, the Associate Referee concluded that it must be a protein or amino acid. This conclusion was further substantiated by the fact that the final filtrate from the procedure showed a surprising amount of nitrogen. This material also tended to clog the filter when the copper oxide was filtered.

Several modifications in the procedure for protein precipitation were tried, as well as several new precipitants, in an attempt to eliminate this interference, but with little success. The Associate Referee plans, as soon as time permits, to do more extensive study on controlling the pH of precipitation, as well as at time of extraction of lactose from the feed sample. One difficulty lies in the fact that during the protein precipitation the solution should never be allowed to go very far on the alkaline side of pH 7.0 or the lactose will, in part at least, be brought down by the heavy metals. It is hoped, however, that a neutral solution may assist in the removal of this interference.

When 5-10 per cent of dry skim milk was put into the middlings, the amount of reducing substance was reduced considerably. This was also the case when the middlings were mixed into a blank feed, and in many cases gave blanks even though the middlings were 25-30 per cent of the mixture. This fact indicates that the interference can probably be eliminated by an improved precipitation procedure.

It will be noted (Table 1) that with the exception of the two samples from Griem, both of which were from the same source of material, the results on the flour middlings average ca. 0.50 per cent as lactose. Several of these samples of middlings when used up to 27 per cent in feed mixtures gave blanks when run through the procedure.

During the year a number of mixed feeds, on which the percentages of lactose were known, were analyzed by the procedure recommended in the 1939 report, with the exception that washed Fleischmann's baker's yeast was used.

In order to show the reliability or possibilities of the method on feed mixtures, the results on some known feeds are shown in Table 2. The percentage of middlings in each sample is also shown.

Table 2 shows that the procedure modified as indicated, in the hands of the Associate Referee and his assistants, gives results that are acceptable. It seems possible that the interference found in the middlings alone is not carried over into a mixed feed. This was the result with all samples of middlings examined except in the case of the two samples that showed the highest reducing sugar values (Table 1). Further work evidently needs to be done on this subject.

TABLE 2.—*Results on known feeds*

SAMPLE NO.	TYPE OF FEED	LACTOSE ADDED per cent	LACTOSE DETERMINED per cent	MIDDINGS per cent
13051	Calf meal	12.50	12.15	17.5
13068	Chick starter	2.50	2.54	20
13069	Chick starter	2.50	2.56	20
13070	Chick starter	None	None	20
13072	Chick starter	2.50	2.55	20
13073	Chick starter	None	None	20
13078	Chick starter	None	.18	20
13079	Chick starter	2.50	2.74	20
13826	Chick starter	2.50	2.33	20
13827	Chick starter	2.50	2.43	20
13828	Chick starter	2.50	2.36	20
13829	Chick starter	None	None	20
"X"	20% mash	None	None	27.5
"X"	20% mash	5*	4.90	27.5
"Y"	Poultry mash conc.	None	None	10
"Y"	Poultry mash conc.	3*	3.10	10
3A	Calf feed	None	None	21
3A	Calf feed	.50*	.60	21
3A	Calf feed	2.50*	2.48	21
4A	Turkey mash	None	None	15
4A	Turkey mash	1.50*	1.43	15

\* Lactose was added in the laboratory at time of analysis.

#### RECOMMENDATIONS\*

It is recommended that further time be granted to investigate:

(1) Effect of using other yeasts than the one indicated; (2) Possible interference brought about by flour middlings; and (3) Possibilities of shortening the procedure.

Further collaborative work is planned as soon as these points are clarified.

#### REPORT ON VITAMIN D FOR POULTRY

By C. D. TOLLE (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Following the meeting of this Association last year there was organized here in Washington the Animal Vitamin Research Council, which had for its primary purpose the outlining and conducting of a program of research on the subject of vitamin D assay with chicks. Because of the detailed nature of this research program, including extensive collaborative studies this year, the Associate Referee planned no A.O.A.C. collaborative study on the tentative chick method for vitamin D assay.

Extensive research studies on the chick method recently completed in

\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 45 (1941).

the Vitamin Division laboratory have been reported by H. W. Loy *et al.*, *This Journal*, 24, 190-196, 432-440 (1941). Results obtained indicate that the use of bone ash data from all birds regardless of weight gives a result as reliable as that obtained by the present tentative method. On the basis of these studies it is recommended\* that in the tentative A.O.A.C. chick method of assay for vitamin D the requirement "that all chicks that weigh 100 grams or less at the end of the assay period be discarded" be deleted from the method.

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No report on hydrocyanic acid in glucoside-bearing materials was given by the associate referee.

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No report on fat in fish meal was given by the associate referee.

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## REPORT ON CAROTENE

By A. R. KEMMERER (Texas Agricultural Experiment Station,  
College Station, Texas), *Associate Referee*

In V. E. Munsey's report last year, *This Journal*, 22, 664 (1939), carotene was determined in a sample of alfalfa leaf meal and a sample of mixed feed by the Peterson-Hughes modification of the Guilbert method, which was adopted as tentative, *Ibid.*, 79. Three methods were submitted for the measurement of carotene concentration in solutions: namely, the Peterson-Hughes spectrophotometric method, which measures the density of absorption at wave lengths of 480, 470, and 450 millimicrons; the Fraps method, which compares the solutions colorimetrically against standard 0.1 per cent potassium dichromate; and the Russell method, which compares the solutions colorimetrically against 0.036 per cent potassium dichromate. Some of the collaborators also determined the concentration of carotene with the photoelectric colorimeter. As a result of this study it was recommended that the spectrophotometer or the 0.1 per cent potassium dichromate standard be used for determining the concentration of carotene in solutions. In recent reports, Shinn, Kane, Wiseman, and Cary<sup>1</sup> and Fraps and Kemmerer, *This Journal*, 22, 190 (1939), have shown that the solutions obtained for the final estimation of carotene by the adopted procedure may contain from 10 to 20 per cent colored impurities. Thus, the carotene content of samples determined by the A.O.A.C. procedure may be too high. Since Munsey's report, the photoelectric colorimeter has come into much wider use and has been improved to a considerable extent.

In the work this year the collaborators were asked to analyze two samples of alfalfa meal—No. 1, alfalfa meal high in carotene, and No. 2, alfalfa

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 49 (1941).

<sup>1</sup> *J. Biol. Chem.*, 119, Proc. 31st meeting, 89 (1937).

meal low in carotene—by the methods given below. A sample of activated magnesium carbonate adjusted so as not to adsorb carotene was sent also, and the collaborators were asked to try the effect of this adsorbent on the crude carotene extracts obtained in the A.O.A.C. procedure. The collaborators that used a photoelectric colorimeter were asked to report if they used a standard to check their instruments, and those using a spectrophotometer were asked to determine the absorption of a 2 cm. depth of 0.02 per cent potassium dichromate at 480, 470, and 450 millimicrons.

## METHODS

The method sent out for extraction of carotene has been adopted tentatively and is published in *This Journal*, 22, 79 (1939). The collaborators were asked to report the carotene obtained by this procedure as crude carotene.

### METHOD FOR PURIFICATION OF CRUDE CAROTENE

#### REAGENTS

*Activated magnesium carbonate.*—Use the preparation enclosed with samples or prepare one as follows: First purify some commercial crystalline carotene. Dissolve 0.1 gram of carotene in 2–4 ml. of  $\text{CHCl}_3$ , precipitate with 20 ml. of methanol, filter, and wash once with methanol. Dry in a vacuum desiccator for not more than 1 hour. Dissolve 20 mg. of the purified carotene in 1–2 ml. of  $\text{CHCl}_3$  and dilute to 100 ml. with petroleum benzin. Dilute an aliquot with petroleum benzin so that a solution that contains 1–2 p.p.m. of carotene is obtained. Determine the exact quantity of carotene in the solution by one of the methods given below.

Place 2.5 grams of U.S.P. light  $\text{MgCO}_3$  in a 2 oz. bottle. Add 50 ml. of the purified carotene solution, stopper tightly, shake vigorously for 1 minute, and centrifuge without removing the stopper. Determine the carotene, and if no carotene is lost, the  $\text{MgCO}_3$  is suitable for use. If more than 1% carotene is lost, thoroughly mix 50 grams of the  $\text{MgCO}_3$  with 10 ml. of distilled water. Test the mixture with the carotene solution. If carotene is still removed, mix in 5 ml. more of water and test again. Add water in 5 ml. portions until the  $\text{MgCO}_3$  no longer adsorbs carotene.

To purify the crude carotene solution, shake a 50 ml. aliquot for 1 minute with 2.5 grams of activated  $\text{MgCO}_3$  in a 2 oz. tightly stoppered wide-mouthed bottle. Centrifuge without removing the stopper. Determine the pure carotene in this solution to 0.1 p.p.m. by one of the methods given below.

#### DETERMINATION OF CAROTENE IN SOLUTION

Determine the crude and pure carotene in the sample by all three of the following methods, if possible: spectrophotometric, photoelectric colorimetric, and visual colorimetric against 0.1%  $\text{K}_2\text{Cr}_2\text{O}_7$ .

(A) *Spectrophotometric method.*—This method was published in *This Journal*, 22, 664 (1939).

(B) *Photoelectric colorimetric method.*—The photoelectric colorimeter must first be calibrated against pure carotene. Use the calibration available, or if the instrument has not been calibrated, purify some crystalline carotene as directed previously, and make up a stock solution containing 200 p.p.m. of carotene. From this solution make up solutions containing 2.0, 1.6, 1.2, 0.8, 0.4, and 0.2 p.p.m. of carotene and obtain the colorimeter reading for these solutions. Plot colorimeter readings against concentration of carotene on arithmetical graph paper. Use this curve for estimating carotene in the sample. To check the photocolormeter make a solution of  $\text{K}_2\text{Cr}_2\text{O}_7$ .

that contains 0.02% of  $K_2Cr_2O_7$ . So adjust the instrument as always to give the same reading for this solution. If the desired reading is not obtained, recalibrate the instrument. Report the filters used in your instrument and method of calibration if different from above and whether or not you use a standard such as 0.02%  $K_2Cr_2O_7$  to check your instrument.

After the photoelectric colorimeter has been calibrated, read the color of the crude and pure carotene solutions and calculate to 0.1 p.p.m. of carotene.

(C) *Visual colorimetric method*.—This method was published in *This Journal*, 22, 79 (1939).

The Associate Referee appreciates the generous cooperation of the following collaborators.

R. O. Brooke and S. W. Tyler, Wirthmore Research Lab., Malden, Mass.

M. W. Taylor, Agricultural Experiment Station, New Brunswick, N. J.

J. C. Fritz, The Borden Company, Elgin Ill.

F. Hafner, Archer-Daniels-Midland Company, Minneapolis, Minn.

O. I. Struve, Eastern States Cooperative Milling Corporation, Buffalo, N. Y.

H. R. Bird, University of Maryland, College Park, Md.

G. C. Crooks, University of Vermont, Burlington, Vt.

V. O. Wodicka, Purina Mills, St. Louis, Mo.

C. O. Gourley, The Beacon Milling Company, Inc., Cayuga, N. Y.

B. L. Oser, Food Research Laboratories, Inc., Long Island City, N. Y.

B. E. Lesley, California Packing Corp., San Francisco, Calif.

W. J. Rudy, Allied Mills, Inc., Peoria, Ill.

E. J. Lease and J. H. Mitchell, Clemson Agricultural College, Clemson, S. C.

#### COMMENTS OF THE COLLABORATORS

*R. O. Brooke and S. W. Tyler*.—Shaking the petroleum benzin extracts of crude carotene with  $MgCO_3$  had little or no effect.

*M. W. Taylor*.—The activated  $MgCO_3$  was tested with purified carotene and found not to adsorb carotene. The photoelectric colorimeter is calibrated against purified carotene at approximately half yearly intervals. Some change has taken place. The readings obtained with a given concentration of carotene are lower at present than they were two years ago when the instrument was new. The suggestion of using 0.02%  $K_2Cr_2O_7$  as a check on photoelectric colorimeter should be worthwhile. The photoelectric colorimeter has considerable advantage over the visual colorimeter in that it can be used on much less concentrated solutions. The carotene procedure submitted does not allow for adequate sampling of green plants.

*J. C. Fritz*.—The photoelectric colorimeter used was checked a number of times against purified beta carotene and was found to hold adjustment fairly well. Several points in the procedure for extraction of the samples were found unsatisfactory. The repeated extraction of the residue with petroleum benzin offers considerable opportunity for oxidation. After about the third extraction, additional extraction removed color that seemed to be xanthophyl rather than carotene. When the carotene extracts were filtered through paper, the paper seemed to adsorb a considerable amount of carotene.

*F. W. Hafner*.—The petroleum benzin solutions of crude and pure carotene were evaporated to dryness and taken up in  $CHCl_3$ . The  $CHCl_3$  solutions were placed in a Pfaltz and Bauer fluorophotometer and the amount of carotene determined.

*O. I. Struve*.—Identical results were obtained when a slightly simplified extraction procedure was followed. The samples were refluxed with alcoholic KOH as directed. The residue was then extracted with successive 25 ml. portions of a mixture

consisting roughly of 115 ml. of petroleum benzin and 10–15 ml. of 95% ethyl alcohol.

*G. C. Crooks.*—The photoelectric colorimeter used was checked against beta carotene at regular intervals.

*V. O. Wodicka.*—The photoelectric colorimeter is calibrated once or twice a year and has not changed in 2 years. In comparing the method of reading the solutions the conclusions are fairly obvious. The spectrophotometer is probably the most accurate instrument and the photoelectric colorimeter is by far the quickest and easiest to use. There is little to be said for the visual colorimeter outside of its applicability where no other instruments are available. In the separation technic a more simplified procedure is recommended. When water is first added to produce a phase separation, shake the mixture vigorously, instead of merely pouring the water in gently. While this slows the first phase separation to some degree, it eliminates subsequent emulsion trouble and reduces the number of water washes necessary to remove alkali. The adsorptive purification of the solutions, using the  $MgCO_3$  furnished with the samples, seemed to be effective, especially on the poor sample. There is a significant increase in the spectrophotometric purity of the solution after adsorption. However, with the knowledge of adsorbents in its present empirical state it would be undesirable to incorporate this step into the official procedure except on an optional basis when knowledge of the pure carotene content is desired. For the most part, considering the rather serious deterioration of carotene in unfavorable storage conditions and the variations in utilization by the animal, the increased accuracy of the determination with the use of adsorbents barely justifies the extra time and labor required to prepare and test the adsorbent at frequent intervals and to carry through the extra steps in the procedure.

*B. E. Lesley.*—Great difficulty has been experienced with the Duboscq colorimeter and consequently it was not used in the test. The amount of carotene in the samples when determined on the spectrophotometer at a wave length of 480 millimicrons was lower than when determined at 470 and 450.

*W. J. Rudy.*—The crude carotene determined by the A.O.A.C. method ran 203.6 p.p.m. for Sample 1 and 56.6 for Sample 2. When diacetone alcohol was used in place of methyl alcohol, the carotene in Sample 1 ran 181.4 p.p.m. and in Sample 2, 50.0 p.p.m.

## DISCUSSION OF RESULTS

The results of this collaborative study for Sample 1 are shown in Table 1 and for Sample 2 in Table 2. From these and from the comments of the collaborators it is evident that the results varied slightly less among the various collaborators when the photoelectric colorimeter was used than when the visual colorimeter was used. For Sample 1 the largest variation from the mean for crude carotene was 18.0 per cent by the visual colorimeter and 12 per cent by the photoelectric colorimeter. For Sample 2 the largest variation from the mean for crude carotene was 24 per cent by the visual colorimeter and 24 per cent by the photoelectric colorimeter.

When the results by the visual colorimeter are compared with results by the photoelectric colorimeter for the collaborators who used both instruments, it is evident that the visual colorimeter gave results that are slightly higher than those obtained by the photoelectric colorimeter. In 1939 Munsey, *This Journal*, 22, 664 (1939), recommended that for accurate work the 0.1 per cent potassium dichromate standard used in the visual method should be checked against pure beta carotene by several

chemists. However, the opinion of this Associate Referee is that the visual colorimeter at its best is not extremely accurate, and it is doubtful whether much can be gained by calibration of the 0.1 per cent dichromate against pure beta carotene.

Since only three of the collaborators ran the samples by the spectrophotometer, definite conclusions as to its merit cannot be drawn from this study. However, in the collaborative study of Munsey in 1939 results varied less by the spectrophotometer than by the visual colorimeter or the photoelectric colorimeter. It has been the experience of the Associate

TABLE 1.—*Results of collaborative study on carotene for Sample 1 (p.p.m.)*

ANALYST	CRUDE CAROTENE			PURE CAROTENE		
	VISUAL COLORIMETRIC	PHOTO- COLORIMETRIC	SPECTRO- PHOTOMETRIC	VISUAL COLORIMETRIC	PHOTO- COLORIMETRIC	SPECTRO- PHOTOMETRIC
1	214.5	206.3		199.0	197.0	
2	200.2			192.0		
3	250.0	240.5		245.0	238.0	
4	221.8	200.7	217.6	223.9	192.5	201.9
5		195.0			185.0	
6	263.0	205.7		255.5	198.0	
7		240.8			225.0	
8	218.8	219.2		220.0	221.5	
9		201.0			196.0	
10	211.3			200.7		
11		220.5	246.5		213.0	216.5
12	203.6					
13				212.0		210.0
Mean	222.9	214.4	232.1	218.5	207.3	207.8

Referee that the concentration of carotene in solution affects the extinction coefficients and that the extinction coefficients used in the A.O.A.C. procedure only hold for limited concentrations.

The collaborators seem to prefer the photoelectric colorimeter to the visual colorimeter or spectrophotometer, even though the spectrophotometer may be more accurate. A photoelectric colorimeter is very rapid and solutions of very low concentration can be read on it. Some collaborators report their photoelectric colorimeters to be quite stable and to need checking only once or twice a year. The experience of the Associate Referee with a photoelectric colorimeter is not so good as this. It has been found necessary to check the photoelectric colorimeter daily against a primary standard of 0.02 per cent potassium dichromate. Usually there is very little variation, but sometimes the colorimeter is off enough to give results that are in error.

In Tables 1 and 2 is also included the quantity of pure carotene in the



samples obtained by shaking the crude carotene solutions with activated magnesium carbonate. Most of the collaborators found that carotene-like materials were removed by the magnesium carbonate. One collaborator examined the crude and pure carotene solutions spectrophotometrically and found the pure carotene solutions to be appreciably purer than the crude carotene solutions. This fact has also been observed by the Associate Referee.

The tentative A.O.A.C. method for carotene determines the quantity of crude carotene in a sample. This crude carotene may not only contain some vitamin A inactive impurities but may also contain other vitamin A active pigments. If yellow corn is run for carotene by the A.O.A.C.

TABLE 2.—*Results of collaborative study on carotene for Sample 2 (p.p.m.).*

ANALYST	CRUDE CAROTENE			PURE CAROTENE		
	VISUAL COLORIMETRIC	PHOTO- COLORIMETRIC	SPECTRO- PHOTOMETRIC	VISUAL COLORIMETRIC	PHOTO- COLORIMETRIC	SPECTRO- PHOTOMETRIC
1	65.5	60.6		63.4	59.5	
2	58.9			55.5		
3	66.0	66.6		66.0	66.9	
4	81.3	57.4	62.6	67.5	50.5	52.0
5		57.0			52.5	
6	77.0	57.3		70.5	52.0	
7		75.3			67.5	
8	62.9	62.9		63.5	63.5	
9		54.7			56.0	
10	58.1			51.6		
11		56.2	62.2		51.1	50.5
12	56.6					
13				62.0		65.0
Mean	65.8	60.9	62.4	62.5	57.7	55.8

method, the carotene will consist of about 50 per cent cryptoxanthin, which has only one-half of the vitamin A potency of beta carotene. Consequently, the A.O.A.C. method for carotene does not give a reliable index of the vitamin A potency of feeds containing yellow corn. The A.O.A.C. method cannot be used for fresh green materials with a high degree of accuracy. Studies on a method for carotene in fresh green materials are being carried out by another associate referee.

In the opinion of the Associate Referee, the A.O.A.C. method for carotene should be limited to dried hays and grasses. The purification process with magnesium carbonate should be used only when it is desirable to know the pure carotene content but not for the purpose of ordinary control work. Carotene in feeds is affected by so many different factors that the extent of impurity creates very little error in feeds assayed for animal feeding.

It is recommended\*—

(1) That the heading "Carotene—Tentative," page 369, *Methods of Analysis, A.O.A.C.*, 1940, be changed to read "Crude carotene in hays and dried plants—Tentative."

(2) That the adsorption method with use of activated magnesium carbonate be studied further, with a view to its adoption as a method for pure carotene.

(3) That study of this subject be continued.

### REPORT ON MANGANESE IN STOCK FEEDS†

By JOHN B. SMITH, *Associate Referee*, and E. J. DESZYCK  
(Agricultural Experiment Station, Kingston, Rhode Island)

This is the third in a series of reports on this method, *This Journal*, 22, 673 (1939); 23, 654 (1940). Previous collaborative results have shown good agreement for feed mixtures, and the only change in methods sent to collaborators this year was an increase in the range of sample weights to allow the use of 5–15 gram charges, for adaptation to feed ingredients with small quantities of manganese. Three samples were analyzed, a chicken mash of the usual constituents, a sample of corn meal, and a sample of rice bran. These feed ingredients represent a wide range in manganese content.

Results by collaborators (Table 1) are consistent, especially those made by analysts experienced in the use of the method. Both ocular and photoelectric colorimeters were used, and there is no evidence of marked or consistent differences from the types of apparatus. Tyler and Hodgman had difficulty in the oxidation of the small amount of manganese in corn, succeeding only with a 20 gram sample and a 45 minute oxidation period, but other collaborators apparently had no such difficulty. R. O. Brooke objects to the restrictions in size of aliquots and flasks, and suggests that solution of the ash be permitted in beakers rather than limited to the dish used for ashing. These pertinent suggestions can be met by simple changes in the method. The method could be simplified if only filtration were used to produce a clear filtrate, but some analysts wish to pipet the aliquot after the precipitate has settled, avoiding filtration. This makes it necessary to adjust the volume and acidity before taking the aliquot. Deszyck finds less frequent formation of manganese dioxide if the solution is near the boiling point when the potassium periodate is added. This cause of off colors and low results is infrequent with low concentrations, but the condition may occur when the quantities of manganese are greater.

Bandemer, Davidson, and Schaible<sup>1</sup> oxidize the manganese to per-

\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 46 (1941).

† Contribution No. 538 of this Station

<sup>1</sup> *Poultry Sci.*, 19, 116 (1940).

manganate directly in the crucible containing the ash, centrifuge, make to volume, and read in a colorimeter, but present several modifications to care for unusual cases. They prefer a 30 per cent solution of periodic acid to dry potassium periodate as the oxidizing agent. Brooke and Tyler in reporting results for the collaborative samples, present equally satisfactory results obtained by extracting the ash in the crucible with only phosphoric acid, dilution with water, and filtration, before developing the color and making to volume. These modifications are simpler than the procedure recommended, but may not be as widely applicable, without precautionary modifications.

TABLE 1.—*Description of samples and collaborative results*  
(p.p.m. of Mn on air-dry basis)

	SAMPLES*		
	1	2	3
E. J. Deszyck	110	135	5
Arthur L. Haskins Pennsylvania State College	103	136	4
F. E. Randall Coop. G. L. F. Mills, Inc.	107	145	6
Stanley W. Tyler	95	136	4†
Russell J. Hodgman Wirthmore Research Lab.	98	135	—
L. S. Walker Vermont Agricultural Experiment Station	109	142	4
Lawrence L. Wiseman University of Missouri	114	160	7
Average	105	141	5

\* Sample 1.—Yellow corn meal, alfalfa meal, flour middlings, fish meal, ground oats, dried skim milk, meat scraps, limestone, salt, cod liver oil, manganese sulfate to supply 70 p.p.m. of Mn.

Sample 2.—Rice bran.

Sample 3.—Corn meal.

† 20 gram sample. Color developed only after 45 min. at temperature of boiling water.

It has been stated in a previous report that this method may not measure manganese combined as acid-insoluble silicates, and for that reason it seems best to label it a method for acid-soluble manganese. By decomposing the ash with hydrofluoric and sulfuric acids, Deszyck finds for Samples 1, 2, and 3 the following results: 110, 151, and 5 p.p.m. By fusion with sodium carbonate, comparable analyses are: 116, 158, and 5 p.p.m. For Samples 1 and 3, a mixed ration and corn meal, these results are not significantly higher than his results for acid-soluble manganese, but there is a gain of approximately 20 p.p.m. for the rice bran.

However, it seems inappropriate to include the portion of the element that can be measured only by such drastic methods of decomposition, for it is unlikely that such manganese is assimilated from the digestive tracts of animals. It is more probable that the acid solvent now used goes too far for exact correlation with assimilability. On the other hand, the possibility of reactions of manganese with siliceous compounds during ashing to form insoluble products may need study. Deszyck oxidized the collaborative samples by wet digestion with sulfuric and nitric acids, and the results were not greater than those by ashing, followed by solution. Possibly more samples should be studied before reaching a conclusion on this point.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the method published in *This Journal*, 22, 78 (1939), and the revision in *This Journal*, 23, 654 (1940), be changed to read as follows:

Ash a weighed sample, 5–15 grams, at dull red heat in a porcelain dish. When cool, add 5 ml. of  $H_2SO_4$  and 5 ml. of  $HNO_3$  to the ash in the dish or to the ash transferred to a beaker with 20–30 ml. of water. Evaporate to white fumes. If carbon is not completely destroyed, add further portions of  $HNO_3$ , boiling after each addition. Cool slightly, transfer to a 50 or 100 ml. volumetric flask, and add a volume of  $H_3PO_4$  soln (8 ml. 85%  $H_3PO_4$ +92 ml. of water) equal to  $\frac{1}{2}$  the volume of the flask (25 or 50 ml.). Cool, make to volume, mix, and filter or let stand until clear. If a 50 ml. flask was used, pipet 25 ml. of clear solution into a beaker or 50 or 100 ml. volumetric flask and add 15 ml. of water. If a 100 ml. flask was used, pipet 50 ml. into a beaker or a 100 ml. flask and add 30 ml. of water. Heat nearly to the boiling point, and with stirring or swirling, add 0.3 gram of  $KIO_4$  for each 15 mg. of Mn present. Compare with the standard  $KMnO_4$  solution in a colorimeter. Calculate p.p.m. of Mn in the sample.

(2) That the method be entitled "Acid-Soluble Manganese in Grain and Stock Feeds."

(3) That with these changes the method be made official (first action).

(4) That the study be continued.

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#### REPORT ON AMMONIACAL AND UREA NITROGEN (FEEDS)

By W. B. GRIEM (State Department of Agriculture, Madison, Wis.),  
under direction of L. S. WALKER (Agricultural Experiment  
Station, Burlington, Vt.), *Referee*

This work was undertaken as an emergency project. Recent research studies have conclusively shown that ruminants can utilize ammoniacal and urea nitrogen as partial substitutes for protein. Apparently the bacteria in the rumen utilize the simple nitrogen forms for protein synthesis for cell structure. These cells are then digested on further passage through

the digestive tract. Of the domestic animals, cattle and sheep and presumably the goat are able to make use of these forms of nitrogen.

If ammoniacal salts and urea become acceptable ingredients for special purpose feeds for these animals it is desirable to have available a method of analysis so that proper labeling can be established. Such a method could also be used for the detection and measure of adulteration of feeds.

A simple procedure was worked out for the determination. It is based on the method for ammoniacal nitrogen in fertilizers and requires no special apparatus other than the regular Kjeldahl equipment. The standard acid and base are those normally used and the deformer is that used in the potash determination in fertilizers. The method follows:

#### AMMONIACAL AND UREA NITROGEN (FEEDS)

##### REAGENTS

- (a) *Standard acid* —II, 19, (a) or (b)
- (b) *Standard alkali*—II, 19, (c)
- (c) *Indicator* —II, 19, (h) or (i)
- (d) *Diglycol stearate solution*.—Dissolve 20 grams of diglycol stearate Tech. in 1 liter of equal parts of benzol and ethyl alcohol (to prevent frothing).
- (e) *Urease solution*.—Prepare a fresh solution by dissolving standardized urease in water so that each 10 ml. of neutralized solution will convert the nitrogen of at least 0.1 gram of pure urea. SUGGESTED STANDARDIZATION PROCEDURE: To determine alkalinity of commercial urease preparation dissolve 0.1 gram in 50 ml. of water and titrate with 0.1 *N* HCl, using methyl red indicator. Add this quantity of 0.1 *N* HCl to each 0.1 gram of urease in preparing the urease solution. To determine the enzyme activity prepare ca. 50 ml. of a neutralized 1% solution. Add different quantities of solution to 0.1 gram samples of pure urea and follow with the enzyme digestion and distillation as directed in the determination. Activity of the urease preparation is calculated from the quantity of this urease solution that converted the urea, thereby permitting complete recovery of the nitrogen by distillation.

##### DETERMINATION

Place 2 grams of sample in an 800 ml. Kjeldahl digestion flask with ca. 250 ml. of water. Add 10 ml. of the urease solution, stopper tightly, and let stand at room temp. 1 hour or at 40° for 20 minutes. Cool at room temp. if necessary. Use more urease solution if feed contains more than 5% urea (ca. 12% protein equivalent). Rinse stopper and neck with a few ml. of water. Add 2 grams or more of MgO (heavy type) and 2 ml. diglycol stearate solution and connect the flask with a condenser by means of a Kjeldahl connecting bulb. Distil 100 ml. of the liquid into a measured quantity of standard acid, and titrate with standard alkali, using cochineal or methyl red indicator.

Collaborative work was undertaken. A dairy feed consisting of 5 parts yellow corn, 5 parts oats, 3 parts wheat bran, 2 parts wheat standard middlings, 2 parts corn gluten feed, 1 part linseed meal, 1 part malt sprouts and 1 part brewers' dried grains was prepared; 1 and 2 per cent of urea was added to portions of this feed by substitution equivalent to 0.456 per cent and 0.912 per cent of nitrogen, respectively, or 2.85 and 5.70 per cent protein equivalent. Portions of the three feeds were sub-

mitted to collaborators. The collaborative results giving individual and average nitrogen and protein equivalents are as follows:

*Collaborative results—Combined ammoniacal and urea nitrogen*

COLL. NO.	SAMPLE I DAIRY FEED		SAMPLE II DAIRY FEED+1% UREA		SAMPLE III DAIRY FEED+2% UREA	
	NITROGEN	PROTEIN EQUIV.	NITROGEN	PROTEIN EQUIV.	NITROGEN	PROTEIN EQUIV.
1	0.045	0.28	0.504	3.15	0.962	6.02
	0.045	0.28	0.504	3.15	0.959	6.00
	Av. 0.045	Av. 0.28	Av. 0.504	Av. 3.15	Av. 0.960	Av. 6.01
2	0.087	0.54	0.512	3.20	0.947	5.92
	0.042	0.26	0.491	3.07	0.968	6.05
	Av. 0.064	Av. 0.40	Av. 0.501	Av. 3.13	Av. 0.957	Av. 5.98
3	0.035	0.219	0.49	3.06	0.924	5.77
	0.035	0.219	0.48	3.00	0.930	5.81
	0.035	0.219	Av. 0.485	Av. 3.03	Av. 0.927	Av. 5.79
	Av. 0.035	Av. 0.22				
4	0.035	0.218	0.495	3.09	0.925	5.78
	0.025	0.156	0.505	3.16	0.915	5.72
	0.025	0.156	0.460	2.88	0.905	5.66
	0.025	0.156	0.455	2.83	0.915	5.72
	0.025	0.156	0.450	2.81	0.925	5.78
	Av. 0.027	Av. 0.17	Av. 0.473	Av. 2.95	Av. 0.917	Av. 5.73
5	0.069	0.43	0.508	3.18	0.943	5.89
	0.069	0.43	0.498	3.11	0.946	5.91
	0.064	0.40	0.512	3.20	0.981	6.13
	Av. 0.067	Av. 0.42	Av. 0.506	Av. 3.16	Av. 0.957	Av. 5.98
6	0.064	0.40	0.448	2.76	0.960	6.00
	0.064	0.40	0.512	3.20	0.976	6.10
	Av. 0.064	Av. 0.40	0.512	3.20	9.960	6.00
			0.512	3.20	0.976	6.10
			0.529	3.30	0.960	6.00
			Av. 0.503	Av. 3.13	Av. 0.966	Av. 6.04
7	0.038	0.24	0.485	3.03	0.954	5.96
	0.030	0.19	0.505	3.16	0.943	5.90
	Av. 0.034	Av. 0.21	Av. 0.495	Av. 3.09	Av. 0.948	Av. 5.93
Av.	0.048	0.30	0.495	3.09	0.947	5.92
Added Urea			0.456	2.85	0.912	5.70
Recovered Urea	Av.		0.447	2.79	0.899	5.62

In the sample of dairy feed without urea additions (Sample I), collaborators reported an average of 0.048 per cent nitrogen or 0.30 per cent protein equivalent of combined ammoniacal and urea nitrogen with mini-

mum and maximum values of 0.17 and 0.42 per cent protein equivalents, respectively. Most of the common commercial feeds give values of the same magnitude most probably caused by the hydrolysis of the amide groups. Malt sprouts, corn gluten feed and meal, dried skimmed milk and buttermilk, and some samples of packing house by-products have been found to give slightly higher values.

In the sample of dairy feed with one per cent added urea (Sample II), 0.456 per cent nitrogen or 2.85 per cent protein equivalent added, collaborators reported an average of 0.495 per cent nitrogen or 3.09 per cent protein equivalent with minimum and maximum values of 2.95 and 3.16 per cent protein equivalent, respectively.

In the sample of dairy feed with 2 per cent added urea (Sample III), 0.912 per cent nitrogen or 5.70 per cent protein equivalent added, collaborators reported an average of 0.947 per cent nitrogen or 5.92 per cent protein equivalent with minimum and maximum values of 5.73 and 6.04 per cent protein equivalents, respectively.

For both samples of feeds with added urea (Samples II and III), from average values reported, the recovery of the added urea was almost perfect when deduction is made for small values obtained in the original dairy feed (Sample I).

Two collaborators reported some difficulty with foaming because they needed to use 500 ml. Kjeldahl flasks. This difficulty was overcome by one of the collaborators by intermittently turning off the electric heaters. One of the collaborators resorted to using 1 gram samples.

Because of the prospect of needing a method for feed control purposes, both for control of adulteration and for legitimate feed formulation, and because of the quite uniform agreement obtained in the collaborative study of this new method it is recommended that the method be given tentative status.

#### COLLABORATORS

- (1) E. F. Budde, The Quaker Oats Co., Chicago.
- (2) Mr. Swift, Agricultural Experiment Station, Lafayette, Ind.
- (3) W. A. Morgan, E. I. du Pont de Nemours & Co., Wilmington, Del.
- (4) W. L. Adams, Rhode Island State College, Kingston, R. I.
- (5) F. F. Hasbrouck, Allied Mills, Inc., Peoria, Ill.
- (6) A. E. Olson, Dept. of Agriculture, Dairy and Food, St. Paul, Minn.
- (7) E. H. LeMense, Wisconsin Department of Agriculture, Madison, Wis.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method for the determination of ammoniacal and urea nitrogen included in this report be accepted as a tentative method.

(2) That the method or modifications be further studied collaboratively.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 24, 46 (1941).

No report on adulteration of condensed milk products and cod-liver oil was given by the associate referee.

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## REPORT ON COSMETICS

By DAN DAHLE (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

In the past little consideration has been given to the problems involved in chemical analysis of cosmetics, and therefore few methods are available in current literature.

The topics assigned at the 1939 meeting of the Association covered rather broad fields. Three associate referees reported, and each has properly chosen to narrow the field of his investigation to a part of the general assignment.

The reports submitted cover the following subjects and topics:

<i>General Subject Assigned</i>	<i>Topic Chosen by Referee</i>
Hair Preparations	Coal-Tar Hair Dyes
Colored Make-Up Preparations	Lipsticks
Facial Preparations	Cold Creams

The details of these projects will be made available by the associate referees themselves in their reports.

## RECOMMENDATIONS\*

It is recommended—

- (1) That the work on cosmetics be continued.
- (2) That more associate referees be invited to participate in the work.
- (3) That methods for analyzing the components of cosmetics, such as moisture, alkalis, lead, arsenic, mercury, beta-naphthol, pyrogallol, etc. be given consideration in addition to studies of cosmetic products, such as depilatories, deodorants, dentrifices, and powders.

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## REPORT ON HAIR PREPARATIONS

By IRWIN S. SHUPE (U. S. Food and Drug Administration,  
Baltimore, Md.), *Associate Referee*

The Federal Food, Drug, and Cosmetic Act of 1938 with its special provisions for coal-tar hair dyes,<sup>1</sup> has created considerable interest and many analytical problems in connection with preparations intended to color human hair. It seems appropriate, therefore, to make hair dyes the subject of the first A.O.A.C. Report on Hair Preparations.

Coal-tar hair dyes belong in two classes. There are those that depend on finished coal-tar colors for their tinctorial power. At present most of

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\* For report of Subcommittee B and action by the Association, see *This Journal*, 24, 50 (1941).

<sup>1</sup>Section 601(a), FD&C Act (1938).



these are designated hair "rinses" or "tints." They usually fail to produce the stability and permanence of color obtainable by the use of the second class, which comprises certain coal-tar intermediates. These intermediates are usually colorless amino compounds. Their oxidation, in contact with the hair, produces the desired dyeing effects. This second class, of which *p*-phenylenediamine is probably the best known member, is of greatest toxicological importance and will be considered in this report.

In order to increase the range of shades and to obtain dyes less toxic than *p*-phenylenediamine, numerous other amino compounds have been proposed for use.

#### I. SEPARATION OF HAIR DYE COMPONENTS BY EXTRACTION WITH IMMISCIBLE SOLVENTS

The separation of the amine constituents of hair dyes may be accomplished by several procedures. Among these may be mentioned the pre-

TABLE 1.—*Solvents suitable for quantitative extractions of amines*

MATERIAL	SOLVENTS	MAY BE EXTRACTED FROM SOLUTIONS MADE ALKALINE WITH:
<p><i>p</i>-Phenylenediamine,  <i>m</i>-Phenylenediamine,            2,5-Diaminotoluene,            2,4-Diaminoanisole,            2,5-Diaminoanisole.</p>	<p>{ Ethyl ether,            Chloroform,            Ethyl acetate</p>	<p>NaHCO<sub>3</sub> or            NH<sub>4</sub>OH or            NaOH</p>
2,4-Diaminodiphenylamine	<p>{ Carbon tetrachloride,            Chloroform,            Ethyl ether,            Ethyl acetate</p>	<p>NaHCO<sub>3</sub> or            NH<sub>4</sub>OH or            NaOH</p>
4-Aminodiphenylamine	<p>{ Petroleum benzin,            Ethyl ether,            Carbon tetrachloride,            Chloroform,            Ethyl acetate</p>	<p>NaHCO<sub>3</sub> or            NH<sub>4</sub>OH or            NaOH</p>
<i>o</i> -Aminophenol	<p>{ Ethyl ether,            Ethyl acetate</p>	<p>NaHCO<sub>3</sub></p>
<i>p</i> -Aminophenol	Ethyl acetate	NaHCO <sub>3</sub>
<i>p</i> -Methylaminophenol	Ethyl acetate	NaHCO <sub>3</sub>
2-Nitro-4-aminophenol	Ethyl acetate	NaHCO <sub>3</sub>

2,4-Diaminophenol—Not extractable with above solvents.

2,5-Diaminobenzene sulfonate—Not extractable with above solvents.

*o*-Aminophenol sulfonate—Not extractable with above solvents.

precipitation of insoluble amine salts of silicotungstic and phosphotungstic acids, the separation of derivatives, and extraction of the free amines with immiscible solvents.

Extraction of the free bases has a rather general applicability and is advantageous for subsequent identification tests. Methods of extraction for some hair dye constituents have been proposed by Griebel and Weisz,<sup>2</sup> who used ether as the immiscible solvent, and by Deshusses,<sup>3</sup> who used ethyl acetate.

A study was made of these and other solvents and their effect on a limited number of amines, commonly used in hair dyes. These amines are retained in aqueous solution by strong mineral acids such as hydrochloric and sulfuric. Table 1 lists the various solvents and alkalies with which quantitative extractions may be obtained.

#### SEPARATION OF DIAMINES FROM AMINOPHENOLS

Mixtures of diamines and aminophenols are often found in hair dyes, particularly in those designed for producing brown and blond shades. The separation of these two classes of compounds can be accomplished since the aminophenols show acidic properties and can be retained by fixed alkali. It was found that a high concentration of alkali is required for the retention of *p*-methylaminophenol.

Table 1 indicates that ethyl ether, chloroform, or ethyl acetate may be used for extracting the diamines from alkaline solutions. In the presence of much alkali, chloroform was not a suitable solvent because of its tendency to form reaction products with the amines. Ethyl acetate, in addition to being gradually hydrolyzed by the alkali, extracted some of the *p*-methylaminophenol. Ether, a neutral organic solvent unaffected by alkali, was found ideal for the extraction. Moreover, the ether solution under the conditions of the method, did not carry over any alkali and did not require washing. The avoidance of the usual requirement of washing the extract prevents loss of material, saves time, and maintains a low volume of alkaline solution, helpful in the subsequent recovery of the aminophenols.

Ethyl acetate was used for the extraction of the aminophenols from the solution made neutral to sodium bicarbonate after removal of the diamines.

#### PROPOSED METHOD

##### REAGENTS

*Sodium hydroxide*.—50% w/v. Dissolve 50 grams of reagent-grade NaOH in sufficient water to make 100 ml.

*Sodium sulfite*.—C.P. powder.

*Sodium bicarbonate*.—C.P. powder.

*Ethyl ether*.—Anhydrous ethyl ether or U.S.P. ether that has been washed with

<sup>2</sup> *Z. Unter. Lebenam.*, 65, 419 (1933).

<sup>3</sup> *Mitt. Lebenam. Hyg.*, 30, 1/2, 10 (1939).

water to remove alcohol. Wash the ether with several ml. of 50% sodium hydroxide solution. Use this alkali-washed ether for all extractions.

*Ethyl acetate*.—Alcohol free or absolute ethyl acetate.

*Acetic anhydride*.—A.C.S. reagent acetic anhydride, less than 0.001% non-volatile residue.

#### EXTRACTION OF DIAMINES

Transfer 5 ml. of an aqueous solution of the sample to a suitable extraction apparatus. (A continuous extractor is recommended.) Add a 2 ml. excess of 50% NaOH solution and about 50 mg. of  $\text{Na}_2\text{SO}_4$ . Extract with ethyl ether. Add 3–4 drops of 50% NaOH to the flask containing the ether solvent. Continue the extraction until the diamines are completely removed (about 3 hours with a fairly efficient extractor). Make a test for complete extraction with a fresh portion of ether solvent, extracting for  $\frac{1}{2}$  hour. (The ether, filtered through cotton, should show no appreciable residue upon evaporation.)

Decant the ether extract through a pledget of absorbent cotton into a tared dish. Use small quantities of  $\text{CHCl}_3$  with the ether to assist in washing the extraction flask and cotton plug. Evaporate the solvent on a steam bath, permitting the last portions to evaporate spontaneously. Dry in a desiccator and weigh the diamines.

#### ALTERNATIVE PROCEDURE

The diamines may be converted to their stable diacetyl derivatives and weighed as follows:

To the filtered ether extract add 0.2 ml. of acetic anhydride for each 0.1 gram of diamine and evaporate to apparent dryness on the steam bath. Evaporate again with a small quantity of alcohol and dry in an oven at  $100^\circ\text{C}$ . for 30 minutes. The residue consists of the diacetyl diamines.

Factors for converting the diacetyl derivatives to the corresponding diamine bases are:

<i>Diacetyl Derivative of:</i>	<i>Factor:</i>
<i>p</i> -Phenylenediamine	0.563
<i>m</i> -Phenylenediamine	0.563
2,5-Diaminotoluene	0.592
2,4-Diaminoanisole	0.622
2,5-Diaminoanisole	0.622
2,4-Diaminodiphenylamine	0.703

#### EXTRACTION OF AMINOPHENOLS

After removal of the diamines, neutralize the alkaline aqueous solution with concentrated HCl. (During the neutralization keep the solution covered with a layer of ethyl acetate to prevent oxidation from the air. Cool in ice water and add the HCl slowly.) When the sample is slightly acid, add an excess of powdered  $\text{NaHCO}_3$ .

If a continuous extractor of suitable size is used, the diamine extraction, the neutralization with  $\text{NaHCO}_3$ , and the subsequent extraction with ethyl acetate may be made in the one apparatus.

If separatory funnels are used, extract with six 20 ml. portions of ethyl acetate. Wash the combined extracts with 5 ml. of water and wash the water with the last 20 ml. portion of ethyl acetate. Filter the extracts through a pledget of cotton into a tared dish. Evaporate the solvent on a steam bath, using a current of  $\text{CO}_2$  to aid evaporation. Permit the last few ml. of solvent to evaporate without heat, dry in a desiccator, and weigh the residue of aminophenols.

EXPERIMENTAL RESULTS ON MIXTURES  
OF KNOWN COMPOSITION

The extraction procedures were applied to various combinations of aminophenols and diamines. Recoveries of the diamines reported in Table 2 are based on the weights of the ether extracts.

TABLE 2.—*Recovery of diamines*

EXP. NO.	CONTAINED:	WT. OF ETHER EXTRACT	RECOVERY OF DIAMINE
	mg.	mg.	per cent
1	<i>p</i> -Methylaminophenol sulfate 300	0.4	—
2	<i>p</i> -Methylaminophenol sulfate 100	0.2	—
	<i>p</i> -Aminophenol 100		
	<i>o</i> -Aminophenol 100		
3	<i>p</i> -Phenylenediamine 100	100.5	100.5
4	<i>m</i> -Phenylenediamine · 2HCl 150	90.8	101.3
5	2,5-Diaminotoluene · 2HCl 150	94.8	101.0
6	2,4-Diaminoanisole · 2HCl 150	99.0	100.8
7	2,5-Diaminoanisole · 2HCl 150	96.1	97.9
8	2,4-Diaminodiphenylamine 100	100.7	100.7
9	<i>p</i> -Phenylenediamine 100	99.6	99.6
	3 Aminophenols 150		
10	<i>m</i> -Phenylenediamine · 2HCl 150	90.0	100.4
	3 Aminophenols 150		
11	2,5-Diaminotoluene · 2HCl 150	94.8	101.0
	3 Aminophenols 150		
12	2,4-Diaminoanisole · 2HCl 150	98.2	100.0
	3 Aminophenols 150		
13	2,5-Diaminoanisole · 2HCl 150	97.0	98.7
	3 Aminophenols 150		
14	2,4-Diaminodiphenylamine 100	98.7	98.7
	3 Aminophenols 150		
15	<i>p</i> -Phenylenediamine 100	188.5	99.4
	<i>m</i> -Phenylenediamine · 2HCl 150		
	3 Aminophenols 150		
16	2,5-Diaminotoluene · 2HCl 150	196.5	102.3
	2,4-Diaminoanisole · 2HCl 150		
	3 Aminophenols 150		
17	<i>p</i> -Phenylenediamine 25	95.0	97.9
	<i>m</i> -Phenylenediamine · 2HCl 25		
	2,5-Diaminotoluene · 2HCl 25		
	2,4-Diaminoanisole · 2HCl 25		
	2,4-Diaminodiphenylamine 25		
	3 Aminophenols 75		

"3 Aminophenols" consisted of equal parts of *o*-aminophenol, *p*-aminophenol, and *p*-methylaminophenol sulfate.

Table 3 shows recoveries of diamines with the alternative procedure, based on the weights of the diacetyl derivatives.

TABLE 3.—*Recovery of diamines by acetylation*

EXP. NO.	CONTAINED:		WT. OF DIACETYL DERIVATIVE	RECOVERY OF DIAMINE
		mg.	mg.	per cent
1	<i>p</i> -Phenylenediamine	100	176.0	99.1
2	<i>m</i> -Phenylenediamine · 2HCl	150	158.1	99.3
3	2,5-Diaminotoluene · 2HCl	150	162.0	102.1
4	2,4-Diaminoanisole · 2HCl	150	152.0	96.2
5	2,5-Diaminoanisole · 2HCl	150	156.5	99.1
6	2,4-Diaminodiphenylamine	100	137.7	96.8
7	<i>p</i> -Phenylenediamine	100	187.8	99.1
	<i>m</i> -Phenylenediamine · 2HCl	150		
8	2,5-Diaminotoluene · 2HCl	150	189.8	98.8
	2,4-Diaminoanisole · 2HCl	150		

Recoveries of total aminophenols are shown in Table 4. These determinations were made on the last three experiments in Table 2 after removal of the diamines.

TABLE 4.—*Recovery of aminophenols*

EXP. NO.	CONTAINED:		AMINOPHENOLS RECOVERED	
		mg.	mg.	per cent
15	<i>p</i> -Methylaminophenol sulfate	50	131	96.6
	<i>p</i> -Aminophenol	50		
	<i>o</i> -Aminophenol	50		
	<i>p</i> -Phenylenediamine	100		
	<i>m</i> -Phenylenediamine · 2HCl	150		
16	<i>p</i> -Methylaminophenol sulfate	50	128	94.4
	<i>p</i> -Aminophenol	50		
	<i>o</i> -Aminophenol	50		
	2,5-Diaminotoluene · 2HCl	150		
	2,4-Diaminoanisole · 2HCl	150		
17	<i>p</i> -Methylaminophenol sulfate	25	66.2	97.6
	<i>p</i> -Aminophenol	25		
	<i>o</i> -Aminophenol	25		
	<i>p</i> -Phenylenediamine	25		
	<i>m</i> -Phenylenediamine · 2HCl	25		
	2,5-Diaminotoluene · 2HCl	25		
	2,4-Diaminoanisole · 2HCl	25		
	2,4-Diaminodiphenylamine	25		

## II. MODIFIED EXTRACTION PROCEDURE FOR A SPECIFIC COMBINATION OF INGREDIENTS

In the analysis of hair dye samples containing ingredients other than amines, special procedures may be required. To illustrate extra steps and to show variations in the use of solvents, a modified procedure is given below. It was used for the separation of resorcinol; 2,5-diaminotoluene; 2,4-diaminoanisole; and *o*-aminophenol in an alcoholic soap solution.

### METHOD

*A. Separation of Fatty Acids.*—Acidify 10 ml. of sample with a 1 ml. excess of concentrated HCl. Extract with three 20 ml. portions of  $\text{CHCl}_3$ . Wash the  $\text{CHCl}_3$  extract with a little water. Filter through cotton into a tared beaker. Dry and weigh the residue of fatty acids.

*B. Separation of Resorcinol.*—After separation of the fatty acids, extract the acid aqueous solution with five 20 ml. portions of ethyl ether. Filter the ether extracts through a pledget of cotton into a tared dish. Evaporate the ether, dry in a desiccator, and weigh the residue of resorcinol.

*C. Separation of the Diamines.*—Neutralize the acid aqueous solution from (B) with powdered  $\text{Na}_2\text{CO}_3$ . (In colored solutions the evolution of  $\text{CO}_2$  serves as an indicator for the presence of excess acid.) Add a small quantity of  $\text{Na}_2\text{SO}_3$  and 1 ml. of NaOH 50% w/v. Extract with five 20 ml. portions of  $\text{CHCl}_3$ . Wash the  $\text{CHCl}_3$  extracts with a little water and filter through cotton into a tared dish. Evaporate on the steam bath, using a stream of  $\text{CO}_2$  to aid evaporation. Permit the last portions of  $\text{CHCl}_3$  to evaporate without heat. Dry in a desiccator and weigh the combined diaminotoluene and diaminoanisole.

*C. 1. Separation of (Para) 2,5-Diaminotoluene from (Meta) 2,4-Diaminoanisole.*—Dissolve the residue of mixed diamines in 15 ml. of 95% ethyl alcohol. Add 1 ml. of (1+1)  $\text{H}_2\text{SO}_4$ . Stir thoroughly and let stand 15 minutes. Filter off the insoluble 2,5-diaminotoluene sulfate and wash with a few ml. of alcohol. Reserve the alcohol solution for the determination of diaminoanisole.

Dissolve the precipitated diaminotoluene sulfate in dilute  $\text{NH}_4\text{OH}$  containing a small quantity of  $\text{Na}_2\text{SO}_3$  and extract with  $\text{CHCl}_3$ . Evaporate the  $\text{CHCl}_3$  extract, dry in a desiccator, and weigh the 2,5-diaminotoluene base.

Make the alcoholic filtrate alkaline with  $\text{NH}_4\text{OH}$  and extract with  $\text{CHCl}_3$ . Wash the  $\text{CHCl}_3$  extract with water to remove most of the alcohol. Evaporate the  $\text{CHCl}_3$ , dry in a desiccator, and weigh the residue of 2,4-diaminoanisole.

*D. Separation of o-Aminophenol.*—To the alkaline aqueous solution from (C) add a slight excess of HCl. Neutralize with  $\text{NaHCO}_3$  and extract with five 20 ml. portions of ethyl ether. Filter the extracts through a pledget of cotton into a tared dish. Evaporate the ether, dry in a desiccator, and weigh the residue of *o*-aminophenol.

The composition of a control sample carried through the above procedure and the recoveries are listed in Table 6.

### DISCUSSION

Exposure of alkaline solutions of these amines to the oxygen of the air causes considerable decomposition even in the presence of sulfites. The use of a continuous extractor is preferable to separatory funnels for extraction since it prevents this oxidation to a large extent by avoidance of

repeated contacts of the sample with the air. A small size Palkin type extractor<sup>4</sup> for solvents lighter than water was found quite satisfactory.

Large quantities of *p*-phenylenediamine, 2,5-diaminoanisole, and 2,4-diaminodiphenylamine separate from strongly alkaline solutions and gradually crystallize. In their crystalline state they are extracted more slowly than from solutions or in amorphous form.

TABLE 6.—*Analysis of control sample*

SAMPLE CONTAINED IN 10 ML.		RECOVERY	
		mg.	per cent
(Alcohol 25%)		—	—
(Sodium Oleate 1%)		—	—
(Na <sub>2</sub> CO <sub>3</sub> 3.8%)		—	—
(Na <sub>2</sub> SO <sub>3</sub> 0.2%)		—	—
Resorcinol	300 mg.	295	98
2,5-Diaminotoluene	300 mg.	291	97
2,4-Diaminoanisole	30 mg.	27	90
<i>o</i> -Aminophenol	60 mg.	56	93

Ethyl ether, a more selective solvent than either chloroform or ethyl acetate, will yield extracts relatively free from colored oxidation products. In badly decomposed samples the ethyl acetate extract of the aminophenols will include some of the oxidation products of the diamines. These aminophenol residues may be purified by vacuum sublimation or by precipitation of the colored products with silicotungstic acid.

The diacetyl derivatives may be fractionally recrystallized by solution in a small volume of glacial acetic acid and addition of petroleum benzin, carbon tetrachloride, or other organic solvent. The para-diamine derivatives are the least soluble in chloroform and ethyl ether.

The melting points of some of the recrystallized derivatives are:

DIACETYL DERIVATIVE OF:	MELTING POINT °C.
<i>p</i> -Phenylenediamine	312–14
<i>m</i> -Phenylenediamine	190– 1
2,5-Diaminotoluene	219–20
2,4-Diaminoanisole	203– 4
2,5-Diaminoanisole	223– 4
2,4-Diaminodiphenylamine	190– 1

#### SUMMARY AND RECOMMENDATION

General extraction properties have been studied for several amino compounds commonly used in hair dyes.

Procedures are suggested for the isolation of diamines and aminophenols from mixtures.

<sup>4</sup>*Ind. Eng. Chem.*, 17, 612 (1925).

It is recommended that specific methods for *p*-phenylenediamine be given collaborative study.

## REPORT ON COLORED MAKE-UP PREPARATIONS

By EDWARD M. HOSHALL (Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The field of colored make-up preparations is extensive and includes practically all cosmetics used in make-up.

A systematic study of such a large group of cosmetics of widely divergent composition in sufficient detail to develop methods of analysis would be impractical, and accordingly it is necessary to narrow this field considerably.

In a more limited sense, therefore, the subject "Colored Make-Up Preparations" may be said to include:

### *Eye Make-Up*

Eyebrow preparations  
Eyelash preparations  
Eye shadow

### *Lip Make-Up*

Lipsticks  
Lip stains  
Other lip preparations

### *Nail Make-Up*

Nail stain  
Nail enamel (lacquer)

### *Face Make-Up*

Rouge  
Sun tan simulatives

The only property common to this variegated group of cosmetics is their color. Coal-tar dyes and mineral pigments are chiefly used, although limited use is still made of animal and vegetable dyes. The physical composition of these products varies from clear liquids through emulsions, creams, and pastes to solids. Their solubilities from the standpoint of bringing them into solution for analysis differ too drastically to be considered collectively.

The topic treated in this report is lipsticks.

## ANALYSIS OF LIPSTICKS

Search of the literature revealed no methods for the comprehensive analysis of the modern lipstick.

From the manufacturers' point of view complete analytical methods are seldom required. They use raw materials conforming to certain specifications, and their testing of the finished product is generally confined to determination of melting-point range, consistency, "indelibility," and organoleptic examination. It is usually much less difficult to duplicate a lipstick of unknown composition by trial and error compounding than by development of a formula after a difficult chemical analysis.

The regulatory chemist will seldom be called upon to make a complete analysis of a lipstick. He may, however, be required to make an examina-



TABLE 1.—*Principal ingredients used in lipsticks*

BASES	APPROXIMATE QUANTITIES USED*
	<i>per cent</i>
<i>Waxes</i> (Stiffening Agents)	
Beeswax (yellow and white).....	
Candelilla wax.....	5-10
Carnauba wax.....	1- 2
Ceresin.....	5-30
Chinese insect wax.....	1-10
Japan wax.....	
Montan wax.....	5-10
Myrtle wax.....	
Ozokerite.....	1-40
Paraffin.....	5-20
Spermaceti.....	5-30
<i>Oils and Fats</i> (Binding, Spreading, and Cutting Agents)	
Almond oil.....	5-10
Benzoinated lard.....	5-15
Butyl stearate.....	5-10
Castor oil.....	5-30
Cholesterin absorption base.....	5-30
Cocoa butter (deodorized).....	1- 5
Corps de jasmin.....	5-10
Diglycol stearate.....	5-10
Glyceryl monostearate.....	5-10
Grape seed oil.....	
Hydrogenated castor oil.....	
Hydrogenated coconut oil.....	
Hydrogenated cottonseed oil.....	
Hydrogenated ground nut oil.....	
Hydrogenated palm kernel oil.....	
Hydrogenated soya bean oil.....	
Lanolin.....	5-10
Lanolin absorption bases.....	5-30
Lard.....	
Lecithin.....	
Mineral oil.....	
Olive oil.....	5-10
Peach kernel oil.....	5-10
Petrolatum.....	1- 5
Sesame oil.....	5-10
Stearic acid.....	1- 5
Triethanolamine stearate.....	1-10
Undecylic acid.....	
Cetyl alcohol.....	
Diethyleneglycol.....	5-30 (3-6)

\* W. A. Poucher, *Perfumes, Cosmetics and Soaps* Vol. 3.

TABLE 1.—*Continued*

BASES		APPROXIMATE QUANTITIES USED*
Diethyleneglycol mono ethyl ether.....		per cent
Styryl alcohol.....		5-10
Sulfated fatty alcohols.....		
Coloring Agents†		
Bromo Acids.....		1-10
FD&C Red No. 3	Erythrosine	
D&C Red No. 21	Tetrabromofluorescein	
D&C Red No. 22	Tetrabromofluorescein NA	
D&C Red No. 24	Tetrachlorofluorescein	
D&C Red No. 27	Tetrachlorotetrabromofluorescein	
D&C Red No. 28	Phloxine B	
D&C Red No. 25	Tetrachlorofluorescein NA	
D&C Yellow No. 7	Fluorescein	
D&C Orange No. 5	Dibromofluorescein	
D&C Orange No. 8	Dichlorofluorescein	
D&C Orange No. 10	Diiodofluorescein	
D&C Orange No. 16	Dibromodiiodofluorescein	
D&C Orange No. 14	Orange TR	
Ext D&C Red No. 4	Dichlorotetraiodofluorescein	
Toners		
Oil-Soluble Coal-Tar Dyes.....		1- 5
FD&C Yellow No. 3	Yellow AB	
FD&C Yellow No. 4	Yellow OB	
FD&C Orange No. 2	Orange SS	
D&C Red No. 17	Sudan III	
D&C Red No. 18	Oil Red OS	
D&C Yellow No. 11	Quinoline Yellow SS	
†Sudan I	Benzeneazo-beta-naphthol	
†Sudan II	Xyleneazo-beta-naphthol	
†Sudan G	Dioxyazobenzene	
Coal-Tar Dyes (as Lakes or Soluble Dye—Toners) .....		5-12
FD&C Red No. 1	Ponceau 3R	
FD&C Red No. 2	Amaranth	
FD&C Red No. 4	Ponceau SX	
FD&C Orange No. 1	Orange 1	
FD&C Yellow No. 5	Tartrazine	
FD&C Green No. 2	Light Green SF	
D&C Red No. 5	Ponceau 2R	
D&C Red No. 6	Lithol Rubin B	
D&C Red No. 8	Lake Red C	
D&C Red No. 10	Lithol Red	
D&C Red No. 14	Lake Red D	
D&C Red No. 19	Rhodamine B	
D&C Red No. 31	Brilliant Lake Red R	

† S. H. Newburger, private communication.

‡ Non-permitted coal-tar dyes.

TABLE 1.—Continued

BASES	APPROXIMATE QUANTITIES USED*
<i>per cent</i>	
<i>Coal-Tar Dyes (Continued)</i>	
D&C Red No. 37	Rhodamine B Stearate
D&C Orange No. 15	Alizarin
Ext D&C Red No. 2	Pigment Scarlet NA
Ext D&C Red No. 5	Rose Bengale TD
Ext D&C Red No. 8	Fast Red S
Ext D&C Yellow No. 1	Metanil Yellow
Ext D&C Yellow No. 5	Franchon Yellow
Ext D&C Orange No. 1	Franchon Orange
†Safranine	Diamidophenyl and Tolytolazonium Chlorides
<i>Mineral Pigments</i>	
Burnt sienna	<i>Penetrating Agents</i> (Also Soothing Agents or Emollients)
(Cadmium red)	
Ferric oxide	Cetyl alcohol
Lamp black (0.01–0.1%)	Cholesterin absorption base
Rouge	Cocoa butter
Ultramarine blue	Lanolin
Yellow ocher	Lanolin absorption base
<i>Other Coloring Agents</i>	
Alkanet root (chiefly historical)	Lecithin
Alloxan (chiefly historical)	Oxycholesterin
Annatto	
Carmine	
<i>Dispersing Agents</i> (Bromo Acid Solvents)	
Butyl stearate.....	5–10
Castor oil.....	10–50
Diethyl phthalate.....	
Lard.....	5–20
Oleyl alcohol.....	3– 5
Sulfated castor oil.....	
<i>Binders</i>	
Diethylene glycol.....	
Diethylene glycol monoethyl ether.....	
Ethylene glycol.....	
Glycerin.....	
Triethanolamine.....	
<i>Preservatives</i> .....0.05–0.5	
Benzoinated lard.....	
Propyl <i>p</i> -hydroxybenzoate.....	
Salicylic acid.....	
Sodium benzoate.....	
<i>Fillers</i>	
Chalk.....	
China clay.....	
Talc.....	Up to 10%
Titanium dioxide.....	2– 6

TABLE 1.—*Continued*

BASES		APPROXIMATE QUANTITIES USED*
		<i>per cent</i>
Zinc oxide.....		2- 6
<i>Perfumes and Flavors.....</i>		1- 2
Alcohols	Ethyl vanillin	
Coumarin	Ionones	
Essential oils	Rose	
Esters	Vanillin	
<i>Miscellaneous</i>		
Anti-blooming agents (lecithin, oxidized cocoa butter).....		0.1-0.5
Anti-oxidants.....		0.1-1.0
Vitamin A.....		Up to 5% of vitamin
"Vitamin F".....		Traces
Proprietary lipstick "improvers".....		

tion for potential cosmetic irritants, coal-tar dyes, and other components that may be found to be of regulatory interest.

As a guide to the chemist engaged in the analysis of lipsticks, Tables 1 and 2 have been compiled from trade publications, formularies, text-

TABLE 2.—*Melting-point ranges of bases used in lipsticks\**

BASE	MELTING RANGE—°C.
Cocoa butter	21.5- 27.3
Lard	36 - 42
Lanolin (anhydrous)	38 - 42
Petrolatum	38 - 54†
Spermaceti	42 - 50
Tallow (beef)	42.5- 44
Mutton suet	45 - 50
Myrtle wax	47 - 48
Paraffin U.S.P. XI	50 - 57†
Diglycol stearate	53 - 54
Japan wax	53.5- 55
Glyceryl monostearate	58 - 60
Ceresin	61 - 78
Beeswax, yellow	62 - 65
Beeswax, white	63 - 70
Ozokerite	65 -110
Candelilla wax	68 - 70
Stearic acid	69 - 70
Montan wax	80 - 86
Carnauba wax	84 - 86
Chinese insect wax	90 - 96

\* Compiled from trade publications, handbooks, catalogs, literature.

† Also available in higher and lower melting ranges.

books, and patents. Typical formulas may also be found in many texts and trade journals.

In view of the many components from which the modern lipstick may be fabricated and of the general impracticability of effecting separations of some of the constituents, the first phase of lipstick analysis was chiefly confined to the separation of the base and color fractions, and to the application of A.O.A.C. methods for such determinations as heavy metals, steam-volatile and unsaponifiable residue, etc. Progress reports on other determinations are also included in this report. To coordinate the determinations discussed in this report with the rather extensive separation scheme required in lipstick analysis, the diagram (Fig. 1) will prove of value. These methods have been used in this laboratory for the analysis of various lipsticks of both known and unknown composition.

## METHODS

### PREPARATION OF SAMPLE

Determine the gross, tare, and net weight of the lipsticks. Remove and weigh the usable portions of the lipstick from at least 3 holders, grind together in a mortar, and preserve from dust and moisture.

### MOISTURE AND VOLATILE MATTER

Transfer 2.0–2.5 grams of the prepared sample to a tared 30 ml. porcelain dish and dry at 100°C. for 2 hours. Report loss in weight as volatile matter. This treatment will remove some of the perfume constituents as well as any water present, for example where an emulsified base or hydrous lanolin is used. If over 6% volatile matter is present, determine water by the xylene distillation method, *Methods of Analysis*, A.O.A.C., 1940, 61, 98.

### I. Preliminary Separation

#### TOTAL BASES

Weigh 2–4 grams of the prepared sample in a tared 30 ml. Gooch (prepared with a 1–2 mm. layer of asbestos) or alundum crucible. Place in an oven at 100°C. until the lipstick just melts and flows over the bottom of the crucible. Transfer to a Dunbar extractor\* and extract with petroleum benzin until a 5 ml. portion of the extract, evaporated on a watch-glass, leaves no visible residue (4–12 hours). Remove the crucible, dry for 1 hour at 100°C., cool, and weigh. The loss in weight is due to "total base," "oil-soluble dyes," and "volatile matter." Retain the crucible with this residue (No. 1) for bromo acid determination.

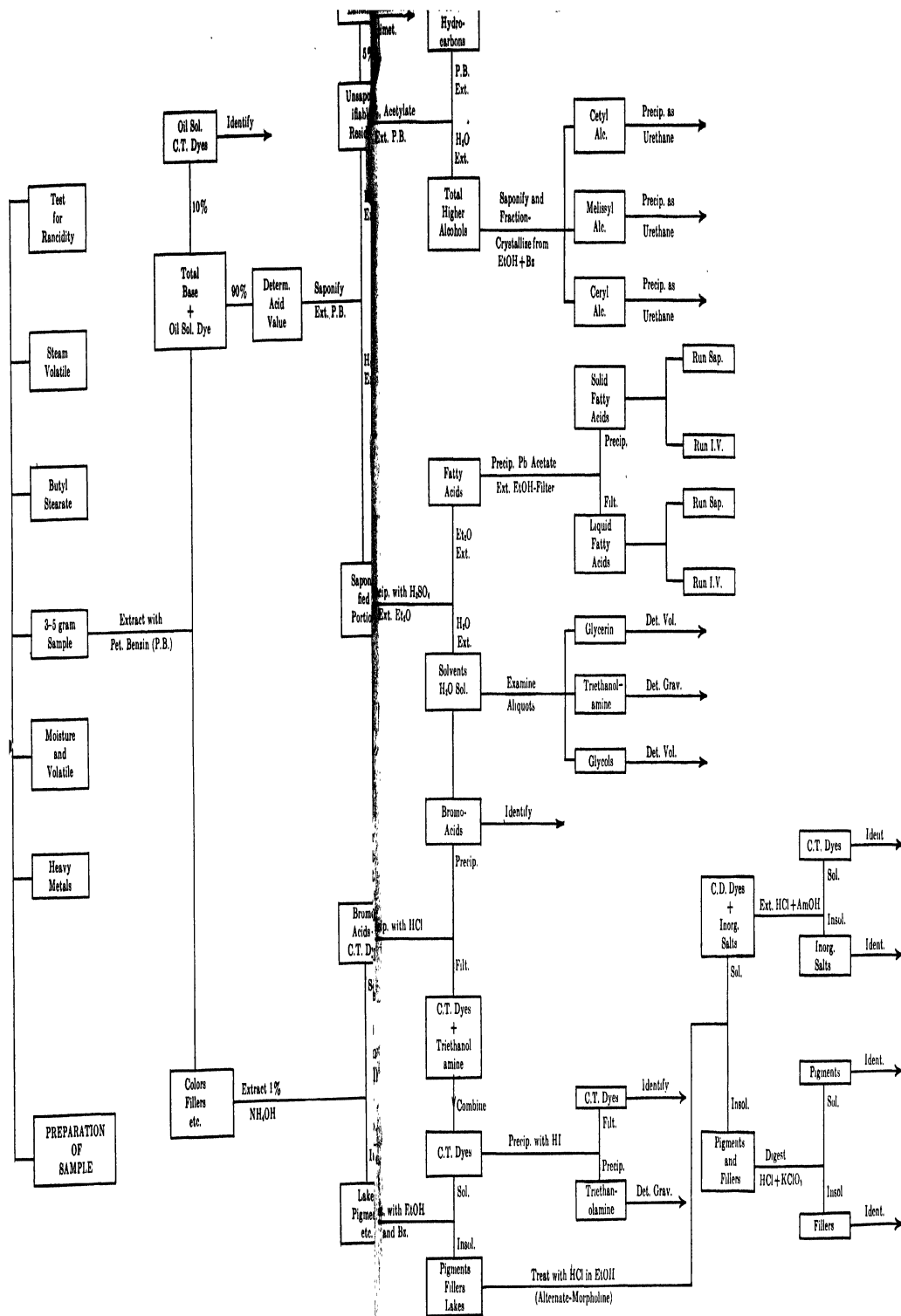
Transfer the extract with boiling petroleum benzin to a tared crystallizing dish, evaporate the solvent on the steam bath, dry the residue at 100°C. for 1 hour, cool, and weigh. The weight of this residue (No. 2) is total base and oil-soluble dyes.

Residue No. 2 may contain a number of the lipstick bases listed in Table 1. The impracticability of a quantitative analysis of a mixture of this nature is apparent. However, certain determinations that will assist in interpreting the general composition follow:

(a) *Oil-soluble Dyes*.—Extract the oil-soluble dyes from Residue No. 2 by the

\* Condenser and cylinder illustrated in Arthur H. Thomas Co. Catalog Ed. 1931, p. 356. Condenser in use contains glass hook from which the crucible is suspended by nichrome wire.









method provided in *Methods of Analysis*, A.O.A.C., 1940, **XXI**, 3 (a), (b), or (c), and identify if practicable by **XXI**, 10-12. (Another solvent often useful in extracting oil-soluble dyes from the base is composed of acetic acid, 82 parts by volume;  $H_2SO_4$ , 9 parts by volume; and water, 9 parts by volume. See Table 1 for oil-soluble dyes used.)

(b) *Unsaponifiable Residue*.—Make this determination on the residue from the oil-soluble dyes (a), either by the method described in *Methods of Analysis*, A.O.A.C., 1940, **XXXI**, 37, or as follows: Remove the petroleum benzin from the lipstick base by evaporation, dissolve in 25 ml. of absolute alcohol, and titrate with 0.1 *N* KOH in absolute alcohol, using phenolphthalein indicator. (Note: This titration can be calculated to the "acid value" of the base.) Add 25 ml. of 2 *N* KOH in absolute alcohol and reflux for 2 hours. Remove the condenser and boil down to ca. 25 ml. Transfer to a 500 ml. separatory funnel with 25 ml. of wash water. Extract with eight 50 ml. portions of petroleum benzin. Combine the petroleum benzin extracts and wash

TABLE 3.—Unsaponifiable matter in lipstick bases

BASE	APPROXIMATE PERCENTAGE
Beeswax.....	55
Candelilla wax.....	75
Carnauba wax.....	55
Ceresin wax.....	100
Chinese insect wax.....	50
Japan wax.....	1
Lanolin.....	50
Montan wax.....	30
Oxycholesterin base.....	75
Ozokerite wax.....	100
Paraffin.....	100
Spermaceti.....	50

with four 25 ml. portions of 10% alcohol, adding the washings to the water-alcohol solution. Transfer the petroleum benzin extract to a tared crystallizing dish, remove the solvent by evaporation, and weigh the residue as "unsaponifiable residue." (The amount of unsaponifiable residue in some of the more common lipstick bases is given in Table 3.) Retain the alcohol-water solution for the following determinations for which methods are to be supplied: Soaps, total fatty acids, glycerin, glycols, and triethanolamine.

The unsaponifiable residue may be examined for lanolin, total hydrocarbons, and higher alcohols (i.e., cetyl, mellissyl, ceryl, etc.). These methods are also to be supplied.

#### BROMO ACIDS

Place the crucible containing Residue No. 1 (cf. above) in a holder in a suction flask, and wash with 10 ml. portions of a hot 1%  $NH_4OH$  solution until the washings are no longer fluorescent. Remove the crucible and dry at 100°C. for 1 hour. The loss in weight is due mainly to alkali-soluble dyes. Retain the crucible with this residue (No. 3) for determination of "toners."

Transfer the ammoniacal washings to a 400 ml. beaker, make acid with HCl, boil gently for 10 minutes, cool, and place in a refrigerator overnight. Filter the precipitate through a tared Gooch crucible, dry at 100°C. for 1 hour, cool, and weigh as

bromo-acids. (The bromo-acids most frequently used in lipsticks are listed in Table 1.) If the filtrate is appreciably colored, extract the dye with amyl alcohol and examine for alkali-soluble dyes. Examine the filtrate for triethanolamine and if present determine as the hydriodide.<sup>1</sup> (Note: A portion of the triethanolamine is usually found in the petroleum benzin fraction, Residue No. 2.)

#### OTHER COAL-TAR DYES (TONERS)

Place the crucible with Residue No. 3 in a Dunbar extractor and extract with benzene until the solvent is colorless. With small quantities of toners it is sufficient to wash into a suction flask with the boiling solvent. Combine and collect the solvent in a tared crystallizing dish, evaporate, dry for 30 minutes at 100°C., cool, and weigh as toners. Retain the crucible with Residue No. 4 for the determination of lakes. (A list of some of the toners used is given in Table 1.)

#### LAKES

Treat Residue No. 4 with a hot solution of 1 part of alcohol + 9 parts of HCl.\* If the solvent, after removal by suction, is colored, continue the extraction until the solvent is nearly colorless. Combine the extracts, evaporate to 20 ml. on the steam bath, and transfer to a separatory funnel. Extract with amyl alcohol and examine the colored extract for coal-tar dyes used in lakes. Combine the aqueous portion with the filtrate from the bromo-acid determination and examine for inorganic salts used as lake precipitants; i.e., sodium, potassium, calcium, barium, strontium, aluminum, magnesium, lead, tin, zinc, etc. Retain the crucible with Residue No. 5 for determination of mineral pigments and fillers.

#### MINERAL PIGMENTS

Place the crucible with Residue No. 5 in a 150 ml. beaker and digest with 10 ml of boiling HCl, adding from time to time 0.5 gram portions of KClO<sub>3</sub>. After digestion is complete, dilute with 50 ml. of water and filter. This treatment will remove the following pigments: Iron oxides (rouges, Indian red, crocus, Ventian red, ocher, sienna, umber, etc.); cadmium red (cadmium sulfoselenide); and ultramarine blue (and green).

#### RESIDUE

The final residue will consist of fillers, substrata for lake colors, or difficultly soluble mineral pigments. Examination may be made for the following: Barium sulfate; silicates (talc, china clay, diatomaceous earth); titanium dioxide; zirconium dioxide; and lampblack.

### II. Supplementary Determinations

#### HEAVY METALS

Use a 5 gram sample of the lipstick and follow the procedure given in *Methods of Analysis*, A.O.A.C., 1940, **XXI**, 27 and 28.

#### STEAM VOLATILE MATTER

Transfer 5-10 grams of the lipstick to a modified Sellier distillation apparatus, *Ibid.*, 168, and distil into a separatory funnel. The steam volatile portion may be examined for essential oils, perfume ingredients, preservatives, etc.

#### BUTYL STEARATE

Determine the total bases (cf. above) on a 3-4 gram sample of the lipstick. Sa-

<sup>1</sup> *Analyst*, 60, 77 (1935).

\* Work by K. A. Freeman in the Color Section of the Cosmetic Division indicates that morpholine is an excellent solvent for many lake colors.

ponify with 20 ml. of 4 *N* aqueous NaOH for 1½ hours, using an efficient condenser to prevent loss of butyl alcohol. Cool the flask in ice water, add 50 ml. of water, and acidify with H<sub>3</sub>PO<sub>4</sub>. Make to 100 ml. in a volumetric flask, filter, and transfer a 50 ml. aliquot to a 200 ml. balloon flask. Proceed with the determination of butyl alcohol described in detail by Werkman and Osburn.<sup>3</sup>

Butyl stearate = butyl alcohol × 4.6.

#### CASTOR OIL

The water-alcohol solution containing the soaps, which remains after the unsaponifiable residue has been removed, may be used to determine castor oil.

The method of Lane<sup>5</sup>, based upon the insolubility of lead ricinoleate in low boiling (28°–30°C.) petroleum benzin, appears to be applicable to the estimation of castor oil in lipsticks. No experimental work has as yet been carried out on this determination.

#### LANOLIN

Lanolin may be determined colorimetrically in an aliquot from the unsaponifiable residue of the lipstick (cf. (b) under Examination of the Total Bases). The details of the method are being worked out.

#### RANCIDITY

Although the recognition of rancidity by the trained chemist by means of taste and odor is not difficult, there are cases where a reliable chemical test may be needed, particularly in highly perfumed lipsticks, when pungent perfumes are used.

The well-known Kreis test<sup>4</sup> and its modifications<sup>5,6</sup> have been studied with regard to their application to the determination of rancidity in lipsticks, and these preliminary studies may be summarized as follows:

In view of the large dye content of lipsticks extraction methods with immiscible solvents yielded fatty mixtures that were not sufficiently free from color (the oil-soluble dyes in particular) to permit the use of the Kreis test. To obviate the interference due to dyes a procedure based on steam distillation of the lipstick was used: 5–10 grams of the lipstick was placed in a modified Sellier distillation apparatus; 5 ml. of 85% H<sub>3</sub>PO<sub>4</sub> was added, and 250 ml. of the steam distillate was collected in a separatory funnel. After filtration, the liquid was saturated with salt and extracted with petroleum benzin. The solvent was transferred to a small separatory funnel, 5 ml. of HCl and 5 ml. of a 0.1% solution of phloroglucinol in ether were added, and the separator was vigorously shaken for 1 minute.

This procedure for rancidity was applied to castor, olive, and sesame oils, which were rancid as judged organoleptically. In every case the Kreis reagent yielded a pink to red color, although the color was not so definite as that obtained when the Kreis test was applied directly to the oil.

Applied to a known lipstick in which no rancid oils were present, the test yielded a pink coloration, owing in part to the coal-tar dyes that were mechanically carried over into the distillate, and to possible reaction with the perfume ingredients. The test was repeated on a similar sample of the known lipstick to which 20 per cent of a rancid olive oil was added. The

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 3, 387 (1931).

<sup>4</sup> *J. Soc. Chem. Ind.*, 26, 579 (1907); See also Allen, *Commercial Organic Analysis*, Vol. II, p. 225. Philadelphia, U.S.A., Blakiston's Son & Co. (1924).

<sup>5</sup> Kreis, *Verhandl. Naturf. Ges. Basel*, 15, 225 (1903–1904); see also Ref. 6.

<sup>6</sup> Aas, J. M., *Fettchem. Umschau.*, 41, 113 (1934).

<sup>7</sup> Kerr, R. H., *Ind. Eng. Chem.*, 10, 471 (1918).

color produced was more intense than that given by the lipstick without the added oil.

Kerr,<sup>7</sup> Powick,<sup>8</sup> and Lea<sup>9</sup> point out that other substances will give a positive Kreis reaction. They mention cottonseed and corn oil, aldehydes, ketones, and essential oils. Many of these may be found in lipsticks and thus render the test of doubtful value.

In their investigation of the Kreis reaction, Taufel and Sadler<sup>10</sup> devised alternative procedures when appreciable coloring matter was present in the oil or fat being tested. Each of the procedures is based on the volatility of epihydrinaldehyde and its reaction with phloroglucinol. These methods will be investigated.

### EXPERIMENTAL

With the exception of the method for the estimation of castor oil, all the methods presented have been applied to commercial samples and to known samples of lipsticks and lipstick bases prepared in this laboratory. The composition of the known samples was as follows:

TABLE 4.—Composition of known samples of lipstick and lipstick base

COMPONENT	LIPSTICK	LIPSTICK-BASE
	<i>per cent</i>	<i>per cent</i>
Beeswax, white	10.0	15.0
Carnauba wax	10.0	10.0
Paraffin, U.S.P.	6.0	6.0
Lanolin, anhydrous	15.5	15.0
Petrolatum, U.S.P.	5.0	5.0
Castor oil, U.S.P.	12.0	14.0
Sesame oil	10.0	15.0
Cetyl alcohol	3.0	3.0
Hydrogenated cotton seed oil	13.5	15.4
D&C Red No. 21	4.0	—
D&C Red No. 12—Barium lake	6.5	—
FD&C Yellow No. 4	1.4	—
Perfume—Oil lavender	0.2	0.2
Oil clove	0.2	0.2
Flavor—Vanillin	0.1	0.1
Preservative (propyl <i>p</i> -hydroxy benzoate)	0.1	0.1
Titanium dioxide	1.0	1.0
Rouge	1.5	—
	100.0	100.0

With the separation scheme previously discussed, qualitative separations were in most cases satisfactory. The methods used for the extraction

<sup>7</sup> *Ind. Eng. Chem.*, **10**, 471 (1918).

<sup>8</sup> *J. Agr. Research* **26**, 323 (1923).

<sup>9</sup> Rancidity in Edible Fats, Food Investigation Spec. Report No. 46, H. M. S. Stationery Office. London (1938).

<sup>10</sup> *Z. Unters. Lebensmittel.*, **67**, 268 (1934):

of the oil-soluble dyes from the base are not entirely satisfactory in that some residual color remains. The work by Freeman on morpholine as a solvent for lake colors (private communication) may overcome some of the difficulties encountered in dissolving the lakes by the present procedure.

Quantitative determinations of bromo-acids, toners, lakes, mineral pigments, fillers, and butyl stearate have yielded generally satisfactory results, but too few determinations have been made. A further report will include this information.

### RECOMMENDATIONS

It is recommended—

- (1) That the study of lip make-up preparations be continued.
- (2) That additional associate referees be appointed for the topics of nail make-up, eye make-up, and face make-up preparations.

### REPORT ON FACIAL PREPARATIONS

By CHARLES F. BRUENING (U. S. Food and Drug Administration,  
Baltimore, Md.), *Associate Referee*

In the attempt to formulate methods for the analysis of facial preparations, the writer thought it desirable to limit this report mainly to one type of facial preparation, cold creams, because they form a very important class of cosmetics and lead all other creams in sales volume. They are also the forerunners of all cosmetic creams, the first cold cream dating back to Galen, a Roman physician of the second century, A.D. They, no doubt, owe their prominence to the fact that they are stable emulsions and are capable of many modifications. With some additions to their basic formulas they may be used as deodorant, all-purpose, cleansing, astringent, and sunburn creams. The methods of analysis presented in this report will deal with the basic cold cream. Modifications of the basic cream will necessarily require modifications in the methods.

Available information will also be presented on the analysis of vanishing cream, which, like cold cream, serves as a prototype for many cosmetic creams, and some of the methods suggested will be applicable to both creams. Vanishing creams are usually oil-in-water emulsions, and cold creams, usually water-in-oil emulsions. By modifying vanishing creams, brushless shaving creams, hand lotions, and "medicated" creams result. For a complete description of the cold and vanishing type creams, their modifications and manufacture, standard text books on cosmetics are available.<sup>1,2</sup>

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<sup>1</sup> F. Chilson, *Modern Cosmetics*, 2nd ed. The Drug and Cosmetic Industry (1938).

Goodman,<sup>2</sup> gives the following as type recipes for cold cream and vanishing cream:

#### *Cold Cream*

- 1—Solid waxes, fats, and grease, e.g., beeswax, paraffin, ceresin, stearic acid, lanolin, lard, petrolatum, 12–20%.
- 2—Liquid oils, e.g., liquid petrolatum, peanut oil, sesame oil, almond oil, 40–60%.
- 3—Water or rose water, 20–35%.
- 4—Binder or emulsifier, e.g., borax, sodium hydroxide, potassium hydroxide, individually or in combinations, 1%.

#### *Vanishing Cream*

	<i>Parts</i>
1—Stearic acid .....	16–24
2—Solid elements, e.g., lard, cocoa butter, tragacanth .....	6
3—Liquid elements: Fatty, e.g., liquid petrolatum, glycerin. not fatty, e.g., water, alcohol .....	75
4—Emulsifiers:	
Borax, KOH, Na <sub>2</sub> CO <sub>3</sub> , or ammonia water .....	1
Triethanolamine .....	1
Cetyl alcohol .....	10
Glycols .....	10
Stearate of triethanolamine .....	10

To test the methods of analysis presented, a cold cream was prepared from the qualitative formula for ointment of rose water, U.S.P. XI, except that mineral oil was substituted for the almond oil. A preservative, *p*-hydroxybenzoic acid, was added. It is believed that this modified formula represents the composition of the majority of cold creams sold in the cosmetic field today. The exact formula follows:

	<i>per cent</i>
Spermaceti .....	8.23
Beeswax .....	12.35
Mineral oil* .....	56.58
Borax (53.76% anhydrous) .....	1.03
<i>p</i> -Hydroxybenzoic acid .....	.10
Water .....	21.71

\* U.S.P. light, viscosity 125, Sp. Gr. at 25°C. 0.8536.

#### METHOD OF PREPARATION

Heat the mixture of beeswax, spermaceti, mineral oil, and *p*-hydroxybenzoic acid in one container to 70°C. In another container, dissolve the borax in the water and heat the solution to 70°C. Pour the aqueous solution into the oil mixture, and stir with a slow-moving, double beater, electric mixer until the mixture is cold. (As water was lost by evaporation during the stirring, the finished batch was weighed to determine the exact water content of the cream.)

#### GENERAL METHODS

(For all creams)

The following methods are presented, accompanied by results (Tables 1–4) on the prepared cream, and by analytical comments relating to both cold and vanishing creams.

<sup>2</sup> H. Goodman, *Cosmetic Dermatology*. McGraw-Hill Book Co. (1936).

*Description of Sample.*—Note color, odor, and other physical characteristics of sample.

*Type of Emulsion.*—Determine the type of emulsion by noting whether the cream will take up oil or water without breaking the emulsion or seriously disturbing the consistency of sample.

This information is of value in determining the type of cream and the probable ingredients, including the emulsifying agents. If a water-in-oil emulsion is heated and phenolphthalein indicator added, soaps or borax will turn the indicator red. A neutral reaction suggests triethanolamine

TABLE 1

TEST	FOUND per cent	THEORY per cent	AV. RECOVERY per cent
Non-volatile material	77.84 77.91	77.81*	100.1
Ash (borates present)	0.47† 0.49	0.55	
Borax (as $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ )	1.06 1.06	1.05	101.0
Water	21.5	21.71	99.0

\* Assuming borax rendered anhydrous.

† Boric acid lost because of insufficient alkali in ash.

glyceryl monostearate, or an absorption base. Usually triethanolamine and glyceryl monostearate creams are oil-in-water and absorption base creams water-in-oil emulsions.

*Non-Volatile Material.*—Dry 2 grams of the sample to constant weight at  $100^\circ\text{C}$ . The volatile material will include water, perfume, alcohol, etc.

*Ash.*—Ignite the non-volatile material at a low temperature and finally at  $600^\circ\text{C}$ . Examine the ash for inorganic constituents.

Borates in the ash would indicate borax as the emulsifying agent. If borax is present, determine it by the A.O.A.C. method for boric acid and borates, *Methods of Analysis, A.O.A.C.*, 1940. Potassium or sodium carbonate would suggest the use of the respective hydroxide or carbonate as the emulsifier. Little or no ash would suggest emulsifiers such as triethanolamine, cetyl alcohol, glyceryl monostearate, or ammonia.

*Water.*—Transfer 5–20 grams of sample to an Erlenmeyer flask, connect to a Dean and Stark distilling tube receiver, and complete the determination by the A.O.A.C. method for moisture in soap, *Ibid*. Read the volume of water obtained and calculate the water content of the cream. Test the water layer for volatile substance such as alcohol and ammonia. To prevent possible charring, use toluene instead of xylene, Shupe, private communication.

Fatty acids, mineral oil, fatty alcohols, waxes, fats, and triethanolamine will dissolve completely in the remaining toluene solution. To detect tri-

TABLE 2

TEST	FOUND per cent	THEORY per cent	AV. RECOVERY per cent
Chloroform extract			
(Dried on steam bath)	77.30, 77.32	77.26	100.1
(Dried at 100°C. for 1 hr.)	76.99, 76.96	77.26	99.6
Mineral oil (A.O.A.C. method)	51.7 53.3	56.58	92.8
Petroleum-benzin-insoluble material (beeswax)	4.91 4.73	12.35	39.0
Unsaponifiable material (hydrocarbons and wax alcohols)	67.24 67.25		
Wax acids	10.35 10.30		
Alcohol-insoluble wax acids	0.95 0.94		
Beeswax acids (alcohol-insoluble wax acids ÷ 0.19)	5.00 4.95		
Beeswax (beeswax acids ÷ 0.45)	11.1 11.0	12.35	90
Soluble wax acids (by difference)	9.40 9.36		
Spermaceti-soluble acids (soluble wax acids minus 0.81 beeswax acids)	5.35 5.35		
Spermaceti (spermaceti-soluble acids ÷ 0.52)	10.3 10.3	8.23	125
Amyl alcohol-hydrochloric acid soluble	4.47 4.66		
Spermaceti (amyl alcohol-hydrochloric acid soluble ÷ 0.48)	9.3 9.7	8.23	115
Hot amyl alcohol-hydrochloric acid- insoluble (hydrocarbons)	60.75 60.55		
Hydrocarbons (by volume measurement)	57.05*	57.44*	99.3
Hydrocarbons (by gravimetric method)	57.47*	57.44*	100.1
Ratio, soluble acids to soluble alcohols	2.05 to 1		
Soluble beeswax acids (soluble acids minus soluble alcohols)	4.81		
Total beeswax acids (soluble beeswax acids ÷ 0.81)	5.94		
Beeswax (total beeswax acids ÷ 0.45)	13.20	12.35	106.9

\* Includes hydrocarbons from beeswax (wax used contained 7% hydrocarbons equivalent to .86% of sample).



ethanolamine qualitatively (Shupe, private communication) pass into the toluene solution dry HCl, filter off the precipitate of the hydrochloride, and identify it by the A.O.A.C. microchemical test. Insoluble in toluene will be glycerol, soaps, gums, borax, starch, and inorganic salts. In the case of the prepared cold cream the toluene-insoluble material contained some of the preservative, *p*-hydroxybenzoic acid, combined as the neutral salt. However, large quantities of the preservative remained dissolved in the toluene. The insoluble material was dissolved in water, the toluene solution extracted with sodium bicarbonate solution, and both aqueous solutions responded to the following test<sup>3</sup>:

*p*-Hydroxybenzoic Acid Test.—To 5 ml. of the solution add 1 ml. of Millon's reagent, and heat on a boiling water bath for 2 minutes. A rose-red color is formed in the presence of 0.1 mg. of *p*-hydroxybenzoic acid.

As this color reaction is given by most phenols, its use is restricted as a specific test for *p*-hydroxybenzoic acid. Other compounds of *p*-hydroxybenzoic acid, such as the methyl, ethyl, and propyl esters, also respond to this test.

*Chloroform-Soluble Material*.—Place 2 grams of sample in a separatory funnel, add 10 ml. of water, acidify with mineral acid, and extract with successive portions of CHCl<sub>3</sub>. Wash the combined CHCl<sub>3</sub> extracts with 5 ml. of water, filter, evaporate on a steam bath until the odor of CHCl<sub>3</sub> cannot be detected, and weigh. Dry at 100°C. for 1 hour and reweigh.

Chloroform is preferred because it is a good solvent for most waxes and fats. Difficulty in extraction is usually experienced, owing to the stability of the emulsion and the emulsifying action of ingredients such as waxes. Small quantities of a mineral acid (hydrochloric acid was used on the prepared cold cream) added to a cream containing borax or soaps, will break the emulsion, permitting rapid and quantitative extraction. With other emulsifiers, the usual methods for breaking emulsions (heating, cooling, addition of solvents, addition of electrolytes) should be tried preliminary to extraction. On the whole, however, if the sample is extracted with liberal portions of the solvent, the emulsion is easily broken, further emulsification formation is minimized, and quantitative extractions obtained. After the preliminary drying on a steam bath, the residue is dried at 100°C. for 1 hour in an oven. Any appreciable loss in weight on this second drying would indicate volatile chloroform-soluble material. With creams prepared with very light mineral oil (viscosity 55–80), a considerable loss might be expected. Physical and chemical constants such as melting point, acid value, saponification value, and iodine value should be determined in order to indicate the probable composition of the chloroform-soluble material.

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<sup>3</sup>Edwards, Nanji, and Hasson, *Analyst*, 62, 178 (1937).

## IDENTIFICATION AND DETERMINATION OF MINERAL OIL, BEESWAX, AND SPERMACETI

In the cold cream prepared, the chloroform-soluble material would consist essentially of mineral oil, beeswax, and spermaceti, with a negligible amount of *p*-hydroxybenzoic acid. The first three ingredients occur together so frequently in many cold creams, lipsticks, and various other creams that it appeared desirable, because of the lack of available methods, to develop procedures for their identification and determination.

Methods for the separation are complicated because these waxes are natural mixtures of various compounds.<sup>4</sup> Beeswax contains as the chief constituent, myricin (myricyl palmitate), melting at 64°C. It also contains cerotic acid,  $C_{26}H_{52}O_2$ , and small quantities of hydrocarbons of the paraffin series. Spermaceti consists chiefly of cetin (cetyl palmitate),  $C_{16}H_{16}O$   $C_{16}H_{31}O$ , with small quantities of cetyl alcohol. Cetin also contains small quantities of lauric, myristic, palmitic, and stearic acids, and also dodecyl, tetradecyl, and octadecyl alcohols. In addition, spermaceti and beeswax resemble each other in that both are esters of the higher alcohols and palmitic acid.

Preliminary experiments were conducted in an attempt to separate one of the compounds from the other two by differences in solubility in various solvents. Mineral oil may be separated from beeswax, spermaceti, and many fats and oils by the A.O.A.C. method for mineral oil in turpentine. This separation depends on the insolubility of mineral oil in warm 38 *N* sulfuric acid; beeswax, spermaceti, and many fats and oils are soluble. The method was tried on 2 grams of the chloroform-soluble material from the prepared cream, and by the use of the specific gravity of the mineral oil the percentage by weight of mineral oil was calculated. The results obtained (Table 1) are low, apparently because some of the mineral oil was emulsified with the sulfuric acid solution. The results indicate that this method may serve to estimate the quantity of mineral oil in a cream, but because of low yield and anticipated interference by many other compounds usually occurring in cosmetic creams, it does not qualify as an accurate quantitative method.

*Petroleum Benzin-Insoluble Material.*—To 5–10 grams of the  $CHCl_3$ -soluble material add 50–100 ml. of petroleum benzin. Filter off the insoluble material and wash it with ca. 50 ml. of petroleum benzin. Dry the insoluble material at 100°C. for 1 hour and weigh.

A part of the beeswax is separated from spermaceti and mineral oil by the petroleum benzin. In this solvent all the spermaceti and mineral oil dissolve and approximately 40 per cent of the beeswax is insoluble. This figure was obtained when a 7.5 gram sample of the chloroform-soluble material and a total of 125 ml. of petroleum benzin were used. As the percentage of beeswax in a sample varies, the relative quantity of insoluble material will no doubt vary, thus restricting this test to a rough quantita-

<sup>4</sup> Wood and Le Wall, U. S. Dispensatory, 22nd. Cent. Ed. J. B. Lippincott Co.

tive approximation. This test should, however, be recognized as qualitative in the sense that if beeswax is present, an insoluble residue will be obtained; if no residue is obtained, beeswax is absent. Physical and chemical constants can be determined on the insoluble residue and, by comparison with those shown in Tables 3 and 4, the identity of beeswax can be ascertained.

TABLE 3

FRACTION	SAMPLE			BEESWAX			SPERMACETI		
	M.P.	ACID VALUE	IODINE VALUE	M.P.	ACID VALUE	IODINE VALUE	M.P.	ACID VALUE	IODINE VALUE
	°C.			°C.			°C.		
Entire Wax				64	19.5	7.1	47	0.5	3.0
Alcohol-insoluble Acids	81.5	134.4	0.7	79.5	132.5	0.8			
Alcohol-soluble Acids	46	212.1	7.2	49.5	203.7	9.5	42.5	228.9	4.7
Amyl Alcohol-Hydrochloric Acid-soluble Alcohols	45.8			79			49.5		
Petroleum Benzin Insoluble Material	71	29.1		72					

The literature showed that mineral oil, spermaceti, and beeswax can be identified and the relative quantities closely approximated by the methods of S. Zweig and A. Taub.<sup>5</sup> In this work, methods were developed for the identification of the more commonly used waxes, including spermaceti, beeswax, carnauba, candelilla, and montan. Cognizance was

TABLE 4

FRACTION	ESTER VALUE	ESTER VALUE ACID VALUE
Beeswax	74.4	3.8*
Petroleum benzin-insoluble material	72.5	2.5
Spermaceti	123.0	Very high

\* Usual variation for beeswax 3.6 to 3.8.

taken of certain allied substances such as cetyl alcohol, rosin, and stearic acid, which might interfere with the analysis. It is noted, however, that compounds containing alcohols and fatty acids such as lanolin may interfere with the proposed procedures. Further investigation is in progress with reference to interfering substances, and this investigation is to be confined to mineral oil, spermaceti, and beeswax alone. Zweig and Taub recognized the fact that even though the common waxes resembled each

<sup>5</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 9 (1940).

other in chemical composition, there were variations in the proportion of acids, alcohols, and hydrocarbons, and differences in chain length of the two components of the esters. Their methods utilize these differences. Through their procedures a practical system of identification is evolved based on the determination of pertinent physical and chemical constants, and on the quantitative separation of waxes into groups of homologous compounds and the determination of the properties of the separated fractions. Table 3 gives the physical and chemical constants on the various fractions.

Interfering substances such as stearic acid, cetyl alcohol, and rosin are removed by an alcohol extraction. Beeswax, spermaceti, and mineral oil are soluble in cold alcohol to a negligible extent, so the above step was omitted and the chloroform extract was treated as directed.

*Unsaponifiable Material and Wax Acids.*—Saponify 10 grams of the fraction insoluble in 95% alcohol at 25°C. by boiling for 2 hours with a mixture of 50 ml. of benzene and 25 ml. of alcoholic KOH (45 grams of KOH in 1000 ml. of absolute alcohol). Transfer the solution to a separatory funnel, add 50 ml. of hot water, draw off the aqueous layer, and wash with three successive volumes of 50, 30, and 30 ml. of warm benzene. Wash the combined benzene solution with a mixture of 2 parts of water to 1 part of the alcoholic KOH solution. Evaporate the benzene and dry the unsaponifiable matter at 100°C. Add to the soap solution 5 ml. of concentrated HCl and shake out the separated acids with warm benzene. Wash the benzene layer twice with equal volumes of water. Evaporate the benzene solution and dry the acids at 100°C.

By this method, the original extract is divided into substances soluble in aqueous alkali after saponification (chiefly wax acids), and into substances insoluble in aqueous alkali, but soluble in benzene (chiefly alcohols and hydrocarbons), known collectively as "unsaponifiable matter." Beeswax contains approximately 55 per cent and spermaceti 48 per cent of unsaponifiable matter, and in the case of the prepared cream, these fractions occurred with the mineral oil in the unsaponifiable matter.

*Alcohol-Soluble and Insoluble Acids.*—Dissolve the acids in 20 parts of hot 95% alcohol. Allow the solution to cool to 25°C. and filter. Two fractions result: (1) Acids soluble in cold alcohol and (2) acids insoluble in cold alcohol.

The acids insoluble in cold alcohol are compounds of high molecular weight with carbon contents from 24 to 34 and melting points above 75°C. Spermaceti does not have any insoluble acids, while 19 per cent (by experimentation on the beeswax used) of the acids of beeswax is insoluble. Thus, in the prepared cream, by dividing the weight of insoluble acids by 0.19, the weight of total acids in beeswax is obtained, and since beeswax consists of approximately 45 per cent wax acids, dividing by this percentage gives the approximate amount of beeswax in the sample.

The acids soluble in cold alcohol are compounds of low molecular weight, chiefly myristic, palmitic, and adjacent homologous acids with

melting points below 51°C. The entire acid fraction of spermaceti and 81 per cent of the acid fraction of beeswax are composed of the lower melting acids and are thus alcohol soluble. If any of the commonly used fats were present in the original sample, the entire fatty acid portion from the fats would be found in the alcohol-soluble fraction. The determination of the iodine value of this fraction would be the best aid in the detection of added fats. In the absence of fats, as in the prepared cold cream, the quantity of spermaceti present is estimated from the weight of the soluble acids. The quantity of spermaceti acids equals the total weight of the soluble acids minus 81 per cent of the beeswax acids, which were calculated from the alcohol-insoluble fraction. Since spermaceti contains 48 per cent unsaponifiable material, dividing the difference found above by .52 gives the approximate quantity of spermaceti.

*Amyl Alcohol-Hydrochloric Acid Fractions.*—Dissolve the unsaponifiable matter from 10 grams of sample in 50 ml. of hot amyl alcohol and add 50 ml. of concentrated HCl (36%). Boil the mixture over an asbestos plate for ca. 5 minutes, stirring it continuously to prevent bumping. (The presence of hydrocarbons is evidenced by an oily layer on the surface of the liquid. When the mixture is cold, a disk of solidified hydrocarbons can be lifted from the lower amyl alcohol-HCl layer). Filter the residual liquid through a Büchner funnel and wash the precipitate twice with 10 ml. of amyl alcohol-HCl mixture. Place the filter paper with the precipitate in an evaporating dish and add boiling water. (The oily layer of wax alcohol rises to the top and solidifies on cooling.) Wash the combined filtrate containing the soluble alcohols in a separatory funnel with hot water until free from HCl. Evaporate or vacuum distill the amyl alcohol. Dry the alcohols at 100°C. and weigh.

This divides the unsaponifiable matter into three fractions: (1) Substances insoluble in hot amyl alcohol-hydrochloric acid mixture (chiefly hydrocarbons); (2) substances soluble in hot amyl alcohol-hydrochloric acid mixture (mainly alcohols of high molecular weight, such as ceryl or myricyl); (3) substances soluble in cold amyl alcohol-hydrochloric acid mixture (mainly wax alcohols of low molecular weight, such as cetyl alcohol).

The wax alcohols are divided into a fraction soluble in cold amyl alcohol-hydrochloric acid mixture and a fraction insoluble in this solvent. Alcohols with more than 22 carbons will be insoluble. The entire unsaponifiable fraction from spermaceti is composed of alcohols soluble in cold amyl alcohol-hydrochloric acid mixture, whereas beeswax yields comparatively small percentages of material soluble in this solvent at 25° C. Therefore, the presence or absence of spermaceti would be indicated by a determination of the quantity of material soluble in the cold amyl-alcohol-hydrochloric acid mixture. In the prepared cream, the weight of soluble material represents approximately 55 per cent of the original spermaceti except for the small quantity of alcohol-soluble material from the beeswax. The quantity of spermaceti calculated from this alcohol fraction should agree with that calculated from the soluble wax acids fraction. With

spermaceti alone, the ratio of alcohol-soluble acids to amyl alcohol-hydrochloric acid soluble acids is approximately 1 to 1. A higher ratio, such as 1.5 or more of soluble acids to 1 part of soluble alcohols, would indicate the presence of beeswax. From the quantity of soluble acids in excess of that required to give a 1 to 1 ratio, the approximate quantity of beeswax can be calculated and its presence verified, if this amount agrees with that calculated from the alcohol-insoluble acids.

The material insoluble in the hot amyl alcohol-hydrochloric acid mixture is mineral oil and hydrocarbons from the beeswax. In treating the unsaponifiable matter with the hot amyl alcohol-hydrochloric acid solvent it was found advantageous to transfer the hot mixture to a separatory funnel, allow to stand until the upper layer was clear, and withdraw the lower layer. The upper layer was washed one time with a small portion of the hot solvent, and after settling, the lower layer was withdrawn and added to the main body of solvent. The upper layer was dissolved in benzene and transferred to an evaporating dish, the solvent was evaporated, and the residue was weighed.

The total hydrocarbons are determined in this insoluble hot amyl alcohol-hydrochloric acid fraction which, apparently, still contains some of the higher alcohols, by the method of Holde<sup>6</sup> for determining mineral oil in wool fat. The method follows:

*Total Hydrocarbons.*—Heat 2–3 grams of the sample with twice the volume of acetic anhydride under a reflux for 2 hours. Transfer the warm contents to a Babcock flask and add warm acetic anhydride until the surface rises well into the graduated neck. Centrifuge while warm and read the volume; if the top layer does not remain liquid, place the flask in warm water until the layer liquefies and then read the volume. If the percentage by weight is desired, determine the specific gravity of the upper layer at a suitable temperature.

An alternative gravimetric method eliminating the specific gravity determination follows: After acetylation of the alcohols, transfer the warm contents of the flask to a separatory funnel, and allow to stand until both layers are separated and clear. If necessary, heat the funnel by placing it in warm water. Withdraw the lower layer and wash the upper layer with a small amount of hot acetic anhydride. Withdraw the washings after separation is complete, dissolve the upper layer in  $\text{CHCl}_3$ , transfer to a suitable dish, evaporate the solvent, dry at  $100^\circ\text{C}$ . until traces of acetic anhydride are removed, and weigh.

In the prepared sample the total hydrocarbons would consist of the mineral oil and a small quantity of hydrocarbons from the beeswax.

#### SUMMARY

Composition is given for typical cold and vanishing creams to serve as an aid in analysis.

Qualitative tests are offered for the determination of the type of emulsion, the identity of the emulsifier and for the preservative.

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<sup>6</sup> Examination of Hydrocarbon Oils and of Saponifiable Fats and Waxes. John Wiley & Sons (1922).

Quantitative tests for the component parts of cosmetic creams are proposed. These tests include non-volatile material, ash, borates, water, and chloroform-soluble material. A typical cold cream was prepared, and the above quantitative determinations were made. All the recoveries are good.

Procedures are given for the analysis of the base of cosmetic creams involving the partial separation of mineral oil from beeswax and spermaceti, and beeswax from mineral oil and spermaceti.

Procedures are also given for the identification of mineral oil, beeswax, and spermaceti when occurring together. These procedures permit a quantitative determination of the mineral oil and a semi-quantitative determination of the two waxes. The identification of these ingredients depends on the quantitative separation involving the isolation of fatty acids of high and low molecular weights, hydrocarbons, and fatty alcohols of high and low molecular weight. Quantitative results obtained on a prepared cream for mineral oil are good, and satisfactory semi-quantitative results for beeswax and spermaceti indicate the method is reliable for the identification of these two substances.

Physical and chemical properties are given for beeswax, spermaceti, and the various derived fractions of each to aid in the identification of these waxes.

The Associate Referee recommends that work on this topic be continued.

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No report on dentifrices and mouth washes was given by the associate referee.

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No report on miscellaneous cosmetics was given by the associate referee.

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## REPORT ON COLORING MATTER IN DRUGS AND COSMETICS

By V. E. GROTLISCH (U. S. Agricultural Marketing Service, formerly of  
U. S. Food and Drug Administration, Washington, D. C.),  
*Associate Referee*

The present year marks the first in which collaborative work on coal-tar colors, other than on the detection and determination of such colors in food products, is included in the agenda of the A.O.A.C. The importance of and need for additional analytical studies on coal-tar colors and their use in drugs and cosmetics were occasioned by the requirement of the Federal Food, Drug, and Cosmetic Act that only certain listed coal-tar colors be permitted for coloring drugs and cosmetics (similar to a like limitation on colors for use in foods). Moreover, no batch of a permitted color or a mixture containing the same can be sold or distributed for such use until after a sample thereof has been submitted to the Food and Drug

Administration and certified as meeting the requirement of the law and regulations as to identity and purity.

The volume of analytical work that developed from this requirement of the law is indicated by the fact that during the Government fiscal year that closed on June 30, 1940, a total of 863 batches of straight or primary coal-tar colors of all kinds, and 2305 batches of repacked colors and various mixtures containing colors from previously certified original batches were examined and certified. Obviously with a limited force of analysts assigned to this work and because of the commercial aspect and mandatory certification of every batch prior to use, the routine certification work took priority over any collaborative studies on improved methods of analysis.

It was possible, however, to devote a limited amount of time to such work, some of which was directly connected with the need for new and suitable methods for colors that had never before been subjected to such rigid specifications as to purity and color strength. For the reasons given, the possibilities of doing worthwhile collaborative work along the line of color analysis have hardly been touched. The reports from the associate referees are submitted as preliminary reports of progress. Much of this field is as yet uncovered by the literature on dye analysis. It is therefore recommended that the several phases of the work on coal-tar color analysis, as covered by the reports, be continued.

## REPORT ON NON-PIGMENT COLORS

By S. S. FORREST (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The regulations governing the certification of coal-tar colors for use in foods, drugs, and cosmetics specify a minimum percentage of pure dye in certified colors.

The quantitative evaluation of pure dye in the majority of water- or alcohol-soluble colors is made by titrating a buffered solution of the color with titanium trichloride. A method was originally proposed by Knecht and Hibbert.<sup>1</sup> The work on the application of this method, with modifications, to these dyes was published in U. S. Department of Agriculture Bulletin No. 1390 (1928) and Supplement No. 1 (1930).

The following method is presented for the triphenylmethane dyes—brilliant blue FCF, fast green FCF, light green SF yellowish, and Guinea green B:

Dissolve 30 grams of sodium acid tartrate in 200 ml. of water in a 1 liter Erlenmeyer flask, boil to expel air, and, under CO<sub>2</sub>, cool to 85°C. Add 100 ml. of dye solution containing 1 gram of dye and titrate under CO<sub>2</sub> at 60°–70°C.

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<sup>1</sup> New Methods in Volumetric Analysis, Longmans, Green & Co. (1918).



For other water-soluble dyes (except erythrosine and amaranth) the method specifies that 125 ml. of water, 15 grams of sodium acid tartrate, and 0.2 or 0.3 gram of dye be used and that the reduction take place at or near the boiling temperature. It was originally assumed that a complete reduction of triphenylmethane dyes would take place only at the lower temperature (60°–70°) and in a relatively dilute solution with a large amount of buffer. Experience in the Color Certification Laboratory, however, has indicated that a more complete, consistent reduction takes place at a temperature at or near the boiling point of the solution and that dilution and the use of a large quantity of buffer are unnecessary.

TABLE 1.—*Analytical results on FD&C Orange No. 1*

COLLABORATOR	MOISTURE	PURE DYE	PURE DYE (WATER-FREE BASIS)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	3.07	91.28	94.17
2	3.17	91.55	94.54
3	3.04	93.20	96.12
4a	3.57	92.99	96.43
4b	3.55	93.07	96.49
5	2.93	93.30	96.11
6	3.44	91.60	94.86
7	3.19	92.09	95.12
8a	3.15	92.75	95.76
8b	3.14	92.15	95.13
9	3.00	94.35	97.26
10	3.31	91.40	94.33
10	—	88.6*	—
11	3.30	91.34	94.46

\* *Note by collaborator.*—We have absolutely no confidence in the dye content of this orange I as obtained in our first titration (88.6%), but it is reported because it may point out a hazard in this determination. The only essential difference in the method employed and our customary method is that in obtaining this lower figure we depended for agitation solely on boiling, and the appearance of the solution near the end point strongly suggested partial decomposition of the dye from local over-heating.

The following modification of the Knecht-Hibbert method was proposed:

Pipet 100 ml. of a stock solution of the dye (1 gram per 100 ml.) into a 500 ml. Erlenmeyer flask. Add 15 grams of sodium bitartrate and 25 ml. of water. Heat to boiling, introduce a stream of CO<sub>2</sub>, and titrate with 0.1 N TiCl<sub>3</sub>, keeping the temperature near the boiling point.

Collaborative analysis was undertaken on one triphenylmethane dye, i.e. brilliant blue FCF, listed as FD&C Blue No. 1,<sup>2</sup> as well as on orange I, listed as FD&C Orange No. 1.<sup>2</sup>

Samples of dyes were submitted to several collaborators, who were asked to determine the pure dye content by both the above methods.

Reports were received from the following collaborators:

<sup>2</sup> Fed. Reg., 4, 1936 (1939).

Bates Chemical Co.

Brooklyn Polytechnic Institute (W. F. Whitmore)

Calco Chemical Co.

H. Kohnstamm & Company

Max Factor & Co.

National Aniline and Chemical Co.

W. J. Stange Co.

Warner-Jenkinson Mfg. Company

O. L. Evenson, J. H. Jones, and S. S. Forrest, Certification Section of this Administration.

In most cases duplicate or triplicate results were submitted, and in some cases more than one analyst in the same laboratory took part in the work. Table 1 lists the averages for each collaborator, in the order the results were received. The average of these results (on a moisture-free basis) is 95.41 per cent, with a standard deviation of  $\pm 1.00$  per cent. Assuming that results falling within  $\pm$  one standard deviation of the mean would be considered acceptable, the pure dye content of this color, calculated on a moisture-free basis, would be 94.41–96.41 per cent. Since none of these results varies more than  $\pm 2$  standard deviations from the mean the need for further statistical work is not indicated.

Reports were received from the collaborators named and also from Kolmar Laboratories on FD&C Blue No. 1.

Table 2 lists the average results in the order they were received.

TABLE 2.—*Analytical results on FD&C Blue No. 1*

COLLABORATOR NO.	MOISTURE	METHOD I*		METHOD II†	
		PURE DYE		PURE DYE	
		AS IS	MOISTURE FREE	AS IS	MOISTURE FREE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	8.78	85.46	93.68	84.17	92.27
2	8.80	85.60	93.85	84.30	92.43
3	8.14	88.30	96.12	83.60	91.00
4a	9.28	87.33	96.26	84.79	93.46
4b	9.20	87.33	96.17	85.08	93.70
5	8.47	87.00	95.05	86.10	94.06
6	9.18	86.30	95.02	85.80	94.47
7	9.05	86.21	94.78	84.88	93.32
8a	8.80	86.10	94.40	85.53	93.78
8b	8.69	85.60	93.74	85.41	93.53
9	4.97	88.30	92.91	86.95	91.49
10	8.60	91.30	99.89	88.92	97.28
11	9.03	87.20	95.85	84.40	92.77
12	9.02	84.56	92.94	83.77	92.07

\* Old Method (Bull. 1390).

† Proposed Method.

The averages, standard deviations, and ranges on a moisture-free basis are—

	Average	Standard Deviation	Range
Method I	95.05	1.81	93.24–96.86
Method II	93.26	1.51	91.75–94.77

Among the individual averages reported in Table 2, one for each method varies from the general average by more than two times the standard deviation. Such a result, having a probability of occurring in a normal distribution less than 5 per cent of the time, could well be considered as due to some abnormality. Hence it may be omitted from the consideration of the acceptable average, standard deviation, and range.

A recalculation with these results omitted would show the following acceptable values on moisture-free basis:

	Average	Standard Deviation	Range
Method I	94.68	1.12	93.56–95.80
Method II	92.95	1.03	91.92–93.98

The results and comments of the collaborators seem to agree on one point: Method I gives higher results than does Method II. A statistical treatment of the individual results submitted (omitted here since only averages are listed in preceding tables) indicates a significant difference in results by Methods I and II. Variance analysis of these individual results also shows:

- (1) For same analyst and same sample: Better checks with Method II.
- (2) For different analysts and same sample: Closer agreement with Method II.

The observation by one collaborator, that the results by Method 2 varied with the time of boiling, is of interest. This laboratory attempted to check this, using a sample of FD&C Blue No. 1 made by this collaborator, and obtained the following data:

Duration of boiling	Pure dye found <i>per cent</i>
Incipient boiling	82.5
Boiling 3 minutes	82.3
Boiling 10 minutes	82.5
Boiling 30 minutes	82.5

During the prolonged boiling precautions were taken to prevent the dye from adhering to and drying on the sides of the heated flask, with possible resultant decomposition. Incidentally, if boiling caused decomposition of the dye, it would be reasonable to expect that the results by Method II should be more variable than those by Method I. The collaborators' data show the reverse to be the case.

It is recommended that further work be done on these colors and that collaborative work be extended to the others of this group.

## REPORT ON LAKES AND PIGMENTS

By G. R. CLARK (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Most of the work on this subject has been done on the development of methods for the determination of the "pure dye" content of pigments. The methods here described have been used in the work of certifying lakes and organic pigments under the Food, Drug, and Cosmetic Act, and have generally given satisfactory results.

## METHODS OF ANALYSIS

The dyes listed in Table 1 may be "stripped" from their substrata by dilute hydrochloric acid and the resultant solution titrated with titanium trichloride, *Methods of Analysis, A.O.A.C.*, 1940, 259-260.

## PROCEDURE

Place a sample containing an estimated quantity of pure dye equivalent to 20-30 ml. of 0.1 N  $\text{TiCl}_3$  in a titration flask and add 100 ml. of HCl (1+19). Boil gently until all dye is dissolved. Dissolve the quantity of buffer indicated in Table 1 in

TABLE 1

NAME OF COLOR	BUFFER USED	QUANTITY
		grams
FD&C (D&C) Blue No. 1	Sodium bitartrate	40
FD&C (D&C) Green No. 1	Sodium bitartrate	40
FD&C (D&C) Green No. 2	Sodium bitartrate	40
FD&C (D&C) Orange No. 1	Sodium bitartrate	25
FD&C (D&C) Red No. 1	Sodium citrate ( $2\text{H}_2\text{O}$ )	20
FD&C (D&C) Red No. 2	Sodium citrate ( $2\text{H}_2\text{O}$ )	20
FD&C (D&C) Red No. 4	Sodium bitartrate	25
FD&C (D&C) Yellow No. 1	Sodium bitartrate	25
FD&C (D&C) Yellow No. 5	Sodium bitartrate	25
FD&C (D&C) Yellow No. 6	Sodium citrate ( $2\text{H}_2\text{O}$ )	20
D&C Orange No. 4	Sodium bitartrate	25
D&C Red No. 5	Sodium bitartrate	25
Ext D&C Yellow No. 3	Sodium bitartrate	25

100 ml. of boiling water, add this to the dye solution, and titrate in the usual manner. Calculate the pure dye content from the factors given in Table 2.

If the dye is not entirely "stripped" from the substrata by the HCl, use additional acid. In this case the quantity of buffer must also be increased.

*D&C Red No. 19.*—Dissolve the dye in HCl (1+19) as directed previously. Dissolve 25 grams of solid sodium tartrate in the acid solution, add 100 ml. of alcohol, and titrate slowly with  $\text{TiCl}_3$  until the color disappears. Add 1 ml. of a 1% solution of Ext D&C Blue No. 1, and titrate back to a blue color with 0.1 N  $\text{Fe}_2(\text{SO}_4)_3$  solution.

To determine the blank, add 1 ml. of the 1% solution of Ext D&C Blue No. 1 to 200 ml. of boiling 50% alcohol containing 25 grams of sodium tartrate. Add an excess of  $\text{TiCl}_3$  and titrate back with standard  $\text{Fe}_2(\text{SO}_4)_3$ .

TABLE 2

NAME OF COLOR	SALT	FACTORS	
		PURE DYE/ML. 0.1 N $\text{TiCl}_3$	0.1 N $\text{TiCl}_3$ /GRAM PURE DYE
		grams	ml.
FD&C (D&C) Blue No. 1	Al.	0.03824	26.15
FD&C (D&C) Green No. 1	Al.	0.03384	29.55
FD&C (D&C) Green No. 2	Al.	0.03824	26.15
FD&C (D&C) Orange No. 1	Al.	0.008408	118.93
FD&C (D&C) Red No. 1	Al.	0.01166	85.76
FD&C (D&C) Red No. 2	Al.	0.01406	71.12
FD&C (D&C) Red No. 4	Al.	0.01131	88.42
FD&C (D&C) Yellow No. 1	Al.	0.00275	363.5
FD&C (D&C) Yellow No. 5	Al.	0.01231	81.24
	Zr.	0.01335	74.91
	Ba.	0.01679	59.56
FD&C (D&C) Yellow No. 6	Al.	0.01061	94.25
	Zr.	0.01130	88.50
D&C Orange No. 4	See D&C Orange No. 1		
D&C Red No. 5	Al.	0.01131	88.42
	Ca.	0.01186	84.32
Ext D&C Yellow No. 3	Al.	0.00916	109.19

1 ml. of 0.1 N  $\text{TiCl}_3$  (net titration) = 0.02435 gram of the aluminum salt of D&C Red No. 19.

1 gram of pure dye = 41.07 ml. of 0.1 N  $\text{TiCl}_3$ .

*D&C Reds Nos. 6-16, inc., 31 and 34.*—Place a sample of the lake containing ca. 0.25 gram of pure dye in a titration flask, add 5 ml. of concentrated  $\text{H}_2\text{SO}_4$ , and mix until all the dye appears to be dissolved. (Add more  $\text{H}_2\text{SO}_4$  if necessary.) Add

TABLE 3

NAME OF COLOR	FACTORS	
	PURE DYE/ML. 0.1 N $\text{TiCl}_3$	0.1 N $\text{TiCl}_3$ /GRAM PURE DYE
	grams	ml.
D&C Red No. 6	0.01021	97.94
D&C Red No. 7	0.01014	98.67
D&C Red No. 8	0.00997	100.30
D&C Red No. 9	0.01111	90.00
D&C Red No. 10	0.01001	99.90
D&C Red No. 11	0.009936	100.65
D&C Red No. 12	0.01115	89.69
D&C Red No. 13	0.01053	94.97
D&C Red No. 14	0.00786	127.28
D&C Red No. 15	0.00900	111.13
D&C Red No. 16	0.00778	128.50
D&C Red No. 31	0.00778	128.50
D&C Red No. 34	0.01104	90.60

carefully, with thorough mixing, 20 ml. of alcohol, and dilute to 100 ml. with alcohol. Heat with stirring until all dye is in solution. Dissolve 20 grams of sodium bitartrate in 100 ml. of boiling water and add 4 ml. of 30% NaOH for each ml. of concentrated  $H_2SO_4$  used in dissolving the dye. Add this buffer to the alcoholic dye solution, stirring rapidly. If the buffer precipitates, add hot water until it redissolves. Titrate the boiling solution in the usual manner until the red color disappears and further addition of  $TiCl_3$  produces no noticeable change.

Calculate the pure dye content from the factors in Table 3.

*Ext D&C Red No. 2.*—Dissolve a sample containing ca. 0.25 gram of the pure dye in  $H_2SO_4$  as directed previously. Add 150 ml. of water and enough 30% NaOH solution to redissolve the dye. Add 15 grams of sodium bitartrate and titrate with  $TiCl_3$  in the usual manner.

1 ml. of 0.1 N  $TiCl_3$  = 0.01639 gram of the barium salt.

1 gram of the barium salt = 61.03 ml. 0.1 N  $TiCl_3$ .

*D&C Red No. 36, Extended on Substrata.*—Place a sample of the lake containing ca. 50 mg. of pure dye in a titration flask, add 100 ml. of glacial acetic acid, and heat on a steam bath until the pigment is dissolved. Add 50 ml. of alcohol, and heat to boiling. Add 10 grams of sodium bitartrate dissolved in 30 ml. of boiling water, and titrate in the usual manner. Add twice as much  $TiCl_3$  as is required to decolorize the solution, and boil for 1 minute after the addition of  $TiCl_3$  has been completed. Add 1 ml. of a 1% solution of Ext D&C Blue No. 1, and titrate back as directed for D&C Red No. 19.

1 ml. of 0.1 N  $TiCl_3$  = 0.00328 gram of D&C Red No. 36.

1 gram of pure dye = 305.13 ml. of 0.1 N  $TiCl_3$ .

*D&C Red No. 35 or Ext D&C Orange No. 1, Extended on Substrata.*—These dyes are not quantitatively reduced by  $TiCl_3$  solution. The pure dye content is calculated from the organically combined nitrogen.

Pure D&C Red No. 35 contains 13.68% nitrogen.

Pure Ext D&C Orange No. 1 contains 15.13% nitrogen.

*D&C Orange No. 15.*—A separate referee is studying this problem.

Acknowledgment is due W. H. King, formerly a member of the Cosmetic Division, for his unpublished work on the back titration method for D&C Red No. 19.

It is recommended—

- (1) That work on the analysis of lakes and pigments be continued.
- (2) That the question of the true chemical nature of lakes be investigated.

## “ REPORT ON ANALYSIS OF COLOR MIXTURES

By O. L. EVENSON (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

This report is of a preliminary nature and will be confined to an outline of the methods used in the Color Certification Laboratory of the Food and Drug Administration for the determination of pure dye in mixtures of coal-tar colors submitted for certification.

The methods applied to food color mixtures have been in use for over

15 years, while those outlined for the drug and cosmetic colors are tentative only and subject to change as better procedures are found.

The application of these methods usually presupposes a knowledge of the formula according to which the mixture is prepared. Such information is submitted by the manufacturer in his request for certification.

### OUTLINE OF METHODS

The methods of analysis vary according to the type of the color mixture on which the pure dye content is to be determined. The following outline attempts to classify the mixtures and indicates a procedure for handling each class.

#### 1. *Mixtures of Water-Soluble Colors*

(a) *Colors reducible with  $TiCl_3$ .*—These comprise the major portion of the food, drug, and cosmetic colors. They are dissolved in water and titrated directly with  $TiCl_3$  in a solution buffered with sodium bitartrate, trisodium citrate, or monosodium citrate according to the kind of color or colors present. According to the reported composition of the mixture the quantity of 0.1 *N*  $TiCl_3$  required to reduce 1 gram of the sample is calculated. From this figure and the amount of  $TiCl_3$  actually required to reduce a known quantity of the sample, the pure dye content of such sample is estimated.

(b) *Mixtures of Water-Soluble Colors.*—One of which does not readily reduce but may be precipitated, filtered off, and weighed.

*Example:* Mixture of FD&C Red No. 2 and No. 3. The FD&C Red No. 3 is precipitated by acidification of the solutions with HCl, filtered off, and weighed. The filtrate containing the FD&C Red No. 2 is titrated as directed in 1(a).

(c) *Mixtures Containing Water-Soluble Colors and Caramel.*—The coal-tar colors are removed by extraction with an immiscible solvent such as amyl alcohol and titrated as directed in 1(a).

#### 2. *Mixtures Containing Oil-Soluble Colors*

(a) *Dry mixtures of the color with flour or rice.*—*Example:* FD&C Yellow No. 3 and No. 4 with flour. These are titrated in 50% alcohol, buffered with sodium bitartrate.

(b) *Colors Dissolved in Vegetable Oils.*—*Example:* FD&C Yellow No. 3 and No. 4 in cottonseed oil. These are titrated in acetic acid. This method is not satisfactory, and the substitution of a spectrophotometric procedure is contemplated.

#### 3. *Mixtures Containing Water-Insoluble Colors or Mixtures of Soluble or Insoluble Colors with Castor Oil.*

(a) *Insoluble Colors.*—*Example:* Mixtures of two or more of the following: D&C Red Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 31, 34, or their lakes. The colors are dissolved in a few ml. of concentrated  $H_2SO_4$ . Water is added, the excess acid is neutralized with NaOH, and the titration is carried out as directed in 1(a).

(b) *Soluble or Insoluble Colors with Castor Oil.*—*Example:* Castor oil with D&C Red No. 7. The oil is saponified with alcoholic NaOH and water is added. Any insoluble color is filtered off. The filtrate containing the soluble dye is titrated as directed in 1(a). The insoluble color is dissolved in  $H_2SO_4$  and treated as directed in 3(a).

(c) *Insoluble Colors with Carbon Black.*—*Example:* D&C Red No. 5 and No. 34 as lakes. The colors are dissolved in  $H_2SO_4$ , alcohol is added, and the carbon is removed by filtration. The filtrate is treated as directed in 3(a).

(d) *Mixtures of D&C Red No. 36 and Insoluble Colors.*—*Example:* D&C Red No. 36 and D&C Red No. 10. About 0.25 gram of the mixture is heated on the steam bath with 100 ml. of glacial acetic acid; 50 ml. of alcohol is added; and the mixture is filtered. The filtrate is titrated with excess  $\text{TiCl}_3$  and back titrated with Ext D&C Blue No. 1 or ferric sulfate. The color on the filter (D&C Red No. 10) is dissolved in  $\text{H}_2\text{SO}_4$  and titrated as directed in 3(a).

(e) *Mixtures of Water-Insoluble Colors with Eosin Colors.*—*Example:* D&C Red No. 21 with one or more of the following: D&C Red Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 31, 34, or their lakes. The mixture is dissolved in  $\text{H}_2\text{SO}_4$  as directed in 3(a), neutralized, and made slightly alkaline. Alcohol is added, the solution is titrated with excess  $\text{TiCl}_3$ , and back titrated with Ext D&C Blue No. 1.

(f) *Mixtures of Water-Insoluble Colors with D&C Red No. 19.*—*Example:* Mixtures of D&C Red No. 19 and any of the colors mentioned in (e). These mixtures are treated as directed in 3(a) except that an excess of  $\text{TiCl}_3$  is added, and back titration with Ext D&C Blue No. 1 or ferric sulfate is carried out.

#### ACKNOWLEDGMENT

The methods for titration of food colors were developed in collaboration with S. S. Forrest, J. A. Kime, and other former and present members of the Color Certification Laboratory. G. R. Clark and W. H. King have done much of the work on the drug and cosmetic colors.

Since the methods for the drug and cosmetic color mixtures are in the tentative stage only, it is recommended that further experimental work be carried out before collaborative work is attempted.

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No report on identification of coal-tar colors was given by the associate referee.

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#### REPORT ON TESTS FOR INTERMEDIATES IN COAL-TAR COLORS

By S. H. NEWBURGER (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The coal-tar color regulations, promulgated under the authority of the Food, Drug, and Cosmetic Act of 1938, list 116 coal-tar colors as harmless and suitable for use. Of these, 18 may be used in food, drugs, and cosmetics; 69 additional in drugs and cosmetics but not in food; and 29 may be employed only in externally applied drugs and cosmetics.

Of the intermediates used in the manufacture of these colors, 36 are potentially dangerous if present in the uncombined form, and the regulations specify the maximum quantities allowed. The term "intermediate," as here used, excludes subsidiary dyes, lower sulfonated dyes, and inorganic reagents used as raw materials.

Methods for the determination of 8 of these intermediates have been published. Seaman, Norton and Mason<sup>1</sup> have described the determination

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<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 345-348 (1940).



of *m*-xylidines and total *o*- and *p*-xylidines in FD&C Red No. 32, and Evenson, Kime, and Forrest<sup>2</sup> the detection of small quantities of 2-naphthylamine, aniline, and *o*-toluidine in FD&C Yellow Nos. 3 and 4. Jablonski, *This Journal*, 22, 771 (1939), has developed a procedure for the estimation of pseudo-cumidine in FD&C Red No. 1. During the past year King, *Ibid.*, 777, and Clark, *Ibid.*, 785, in the Color Certification Section of the U. S. Food and Drug Administration, have published methods for quinaldine and for 1,4-dihydroxy-anthraquinone.

In addition to its published work the Color Certification Section has developed tentative methods for 15 intermediates, among which are tobias acid and  $\beta$ -naphthol. There still remain 13 intermediates on which work is to be done. Some of these are *p*-aminophenol, 2-nitro-*p*-toluidine, and diethyl-*m*-aminophenol.

In general, the analyst must isolate the intermediate from the dye and estimate the amount present. Ordinarily the intermediate is separated by extraction, steam distillation, or sublimation. The method used to isolate a given intermediate varies with the color in which it is used. Thus the extraction of aniline from an oil-soluble color differs from its extraction from a water- or spirit-soluble color. The quantitative estimation is usually done by gravimetric, volumetric, or colorimetric methods. Colorimetric methods are most feasible for the majority of intermediates, in particular, primary amines. It is a relatively simple matter to diazotize and couple these amines with some suitable sulfonic acid.

In collaboration with J. H. Jones of the Color Certification Section, the Associate Referee worked on Lake Red C Amine (2-chloro-5-toluidine-4-sulfonic acid), the intermediate found in D&C Red Nos. 8 and 9.

The following tentative method is offered:

#### REAGENTS

*Barium chloride*.—2% solution.

*Sodium nitrite*.—0.001 *M* solution.

*H-acid*.—0.1% aqueous solution of 1-amino-8-naphthol-3,6-disulfonic acid.

#### DETERMINATION

Weigh a 1 gram sample of powdered dye into a 300 ml. tall-form beaker. Wet with 5 ml. of alcohol (1+5) and add 95 ml. of the BaCl<sub>2</sub> solution. Boil for 10 minutes, cool, and filter. Reserve the filtrate. Return residue and paper to the beaker and re-extract with 95 ml. of boiling water for another 10 minutes. Cool, and filter. Combine the filtrates, acidify with 2 ml. of HCl, and extract with four 30 ml. portions of benzene. Wash the combined benzene extracts with 10 ml. of water, and add this washing to aqueous solution. (Reserve the benzene solution for the determination of  $\beta$ -naphthol.)

Transfer the aqueous solution into a 200 ml. volumetric flask, and dilute to mark. Pipet a 10 ml. aliquot into a 100 ml. test tube, add 1 drop of HCl, cool to 5°C., and diazotize for  $\frac{1}{2}$  hour with 3 ml. of the NaNO<sub>2</sub>. Buffer with excess solid sodium acetate, add 1 ml. of the H-acid, and let couple in the cold for 10 minutes.

<sup>2</sup> *Ind. Eng. Chem., Anal. Ed.* 9, 74-75 (1937).

Remove from the cooling bath, and allow to warm to room temperature for another 20 minutes. Dilute to 50 ml., and compare with standards containing 0.05, 0.1, and 0.15 mg. of Lake Red C Amine, prepared at the same time as the sample and containing an equivalent quantity of the BaCl<sub>2</sub>.

### DISCUSSION

A specially purified D&C Red No. 8 was prepared by wetting the dye with alcohol and extracting it twice with boiling water. This sample, dried at 135°C. and powdered, contained only a slight trace of Lake Red C Amine (as determined by the proposed method).

Known quantities of the amine, dissolved in HCl (1+9), were added to 1 gram aliquots of this sample and the recoveries determined by the proposed method.

The amine was added in one of the following ways:

- (1) To a water suspension of the dye;
- (2) To an alcoholic paste of the color; and
- (3) To the dry color, which was then evaporated to dryness on the steam bath and powdered.

Of these, (3) corresponds most closely to the manner in which the determination would be applied. Table 1 shows the results.

TABLE 1.—*Recoveries of the intermediate*

AMOUNT ADDED	RECOVERIES	METHOD OF ADDITION
mg.	per cent	
0.5	85	(2)
0.5	75	(3)
1.0	80	(2)
1.0	80	(3)
2.0	100	(1)
2.0	80	(2)
2.0	80	(3)
3.0	80	(2)
3.0	75	(3)
4.0	100	(1)

The data in the table show that when the amine was added by Method (1) or (2) the recoveries were better than when Method (3) was used. This seems to indicate that a more efficient water extraction is needed.

It is recommended that work on intermediates in coal-tar colors be continued.

### REPORT ON SPECTROPHOTOMETRIC COLOR TESTING

By RALPH W. STEWART (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The Color Certification Section has available an automatic recording photoelectric spectrophotometer. This instrument automatically records

the photometric characteristics of solutions or solids in a wave length range of 400–750 m $\mu$ . These data can be recorded as transmission, transmittancy, reflection, or reflectance, or these values multiplied by five. The data can also be recorded as density (log of transmittancy) or as extinction (negative log of transmittancy). The time required to draw a

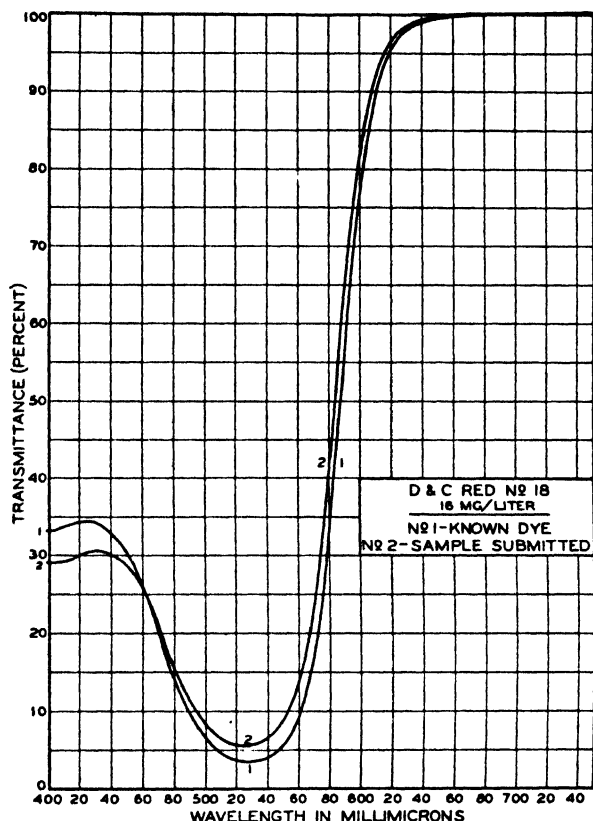


FIG. 1.—TRANSMITTANCY CURVES OF TWO SAMPLES OF D&C Red No. 18.

complete spectral curve on this instrument is  $2\frac{1}{2}$  or 5 minutes, depending on the gear used.

The primary function of this section at present is to certify each batch of coal-tar color intended for use in food, drugs, or cosmetics, and this apparatus has proved to be almost indispensable in this work.

One example of the use of this apparatus is that of proving the identity and determining the pure dye content of primary colors submitted for certification. Figure 1 shows the transmittancy curves of two samples of D&C Red No. 18 (xylylazoxylylazo-beta-naphthol). Curve 1 is a sample of the dye which, after extensive investigation in this laboratory, was found to have a pure dye content of 98.0 per cent and very small quan-

tities of colored impurities. Curve 2 is a commercial sample of this dye as submitted for certification. The curves are parallel throughout the visible spectrum except between 400 and 460  $m\mu$ . This absorption of the unknown in the region of the blue indicates the presence of a yellow colored impurity. By a chromatographic separation the presence of this yellow impurity was definitely proved and the yellow compound tentatively identified as xylylazoxylene, an intermediary product formed in the preparation of the dye.

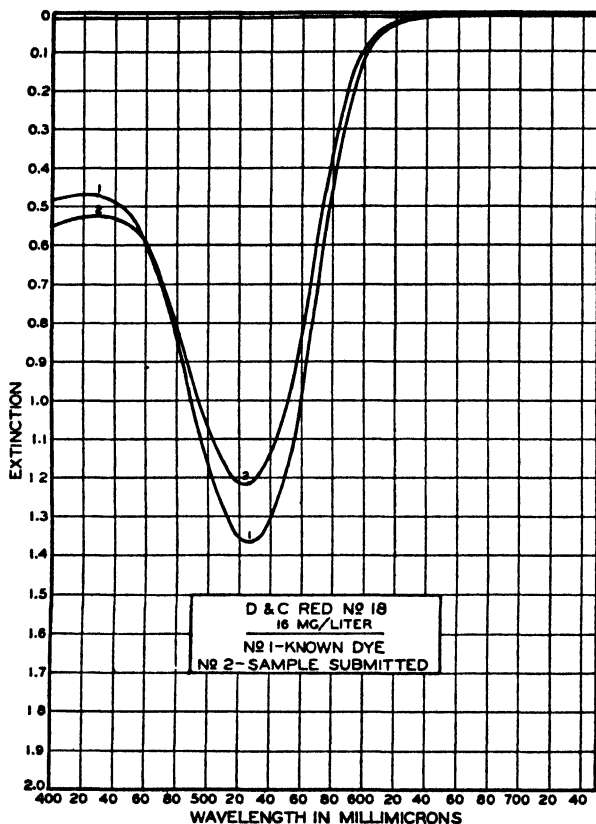


FIG. 2.—EXTINCTION CURVES OF SAME SOLUTIONS SHOWN IN FIG. 1.

Figure 2 shows the extinction curves on these same solutions. The absorption maxima lie between 520 and 530  $m\mu$ . The extinction ratio is determined in this region, because any error in reading the wave length here will result in an insignificant error, whereas if the ratio is determined on the "steep" part of the curve, any error in reading the wave length becomes quite significant.

The ratio of extinction of the unknown dye to the known dye at 525  $m\mu$  is 1.215/1.365, or 0.890, indicating that the unknown solution contains

89.0 per cent as much pure dye as the known solution, or 87.2 per cent pure dye ( $89.0 \times 0.98$ ).

Another example of the use of this instrument is in the analysis of dye mixtures. A sample containing FD&C Orange No. 2 and D&C Yellow No. 11 mixed with stearic acid was analyzed by a procedure that included saponification of the stearic acid, extraction of the dyes with ether from the sodium stearate, evaporation of the ether, and determination of the total nitrogen in the dye residue by the Kjeldahl method. This procedure

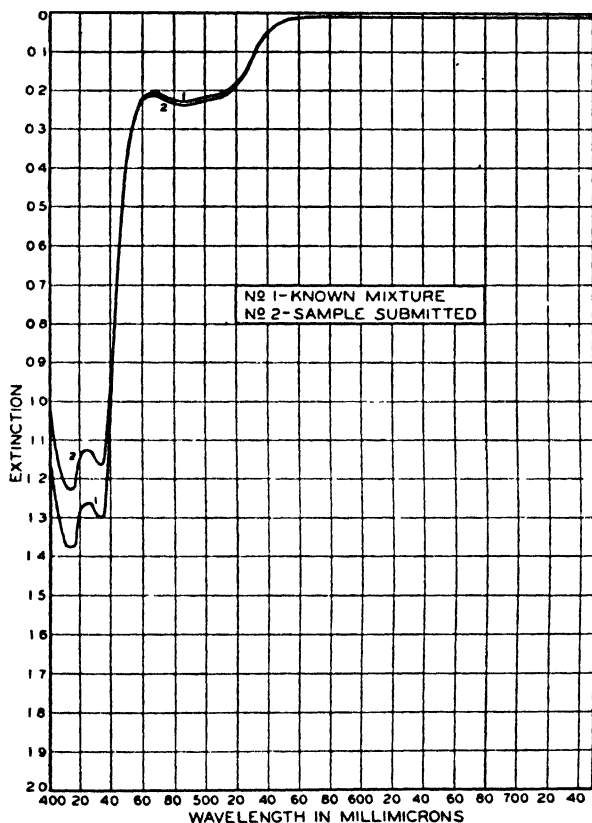


FIG. 3.—EXTINCTION CURVES OF ORIGINAL BATCH AND A SUBSEQUENT BATCH OF COLOR MIXTURE.

required about 10 hours, but gave a nitrogen figure very close to the theoretical. A known mixture of the dyes and stearic acid was then prepared and transmittancy curves of the two mixtures were drawn. The curves were identical and subsequent samples from other batches of this mixture are now checked by simply comparing the curves obtained on them with the known curve. (This comparison requires only about 30 minutes and effects a saving of  $9\frac{1}{2}$  hours on each determination.)

Figure 3 shows the extinction curves of the original batch and a subsequent batch. The curves indicate that the latter batch contained slightly less of the yellow dye and slightly more of the orange dye than did the original.

Another use to which the spectrophotometer has been applied is the estimation and identification of dyes used in hair rinses. A commercial sample of hair rinse showed the presence of three dyes when it was examined by extraction, chromatography, wool dyeing, and spot testing.

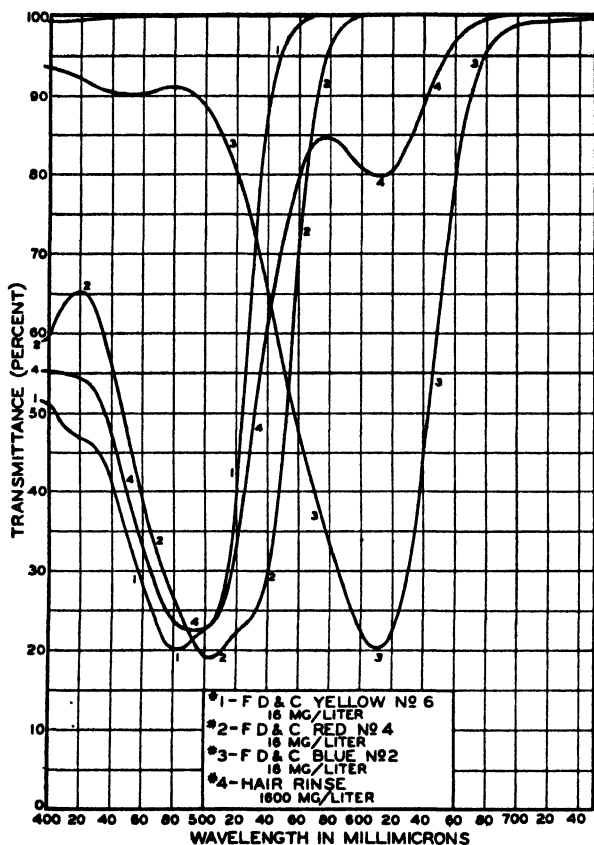


FIG. 4.—TRANSMITTANCY CURVES OF THREE KNOWN DYES AND A COMMERCIAL HAIR RINSE.

The dyes indicated were FD&C Red No. 4, FD&C Yellow No. 6, and FD&C Blue No. 2.

Figure 4 shows curves of these dyes and also a curve of the hair rinse. The curves show definitely that the rinse contains these three dyes and that no other dyes are present. From the extinction values of these curves the percentage of dye in the rinse was found to be 1.08, of which 0.15 per

cent was FD&C Blue No. 2 and the remaining 0.93 per cent was the mixture of FD&C Red No. 4 and FD&C Yellow No. 6. This amount of dye would require for its reduction 0.83 ml. of 0.1 *N* titanium trichloride per gram of dye. A 5 gram sample of the dye when titrated gave an equivalent of 0.78 ml. of 0.1 *N* titanium trichloride per gram, equivalent to 1.02 per cent of pure dye, an actual difference of only 0.06 per cent of dye as determined by the two methods.

It is recommended that the work on spectrophotometric color testing be continued.

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No report on micro methods for coal-tar color analysis was given by the associate referee.

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#### ERRATUM

In the Report on Tests for Pasteurization of Dairy Products, by F. W. Gilcreas, Associate Referee, published in *This Journal*, 24, 559-574 (1941), the numbers prefixed to the names of the cooperating laboratories on pages 560 and 569-570 bear no relation to the numbers of the laboratories as they appear in Tables 1-6, inc.

## CONTRIBUTED PAPERS

### FACTORS INFLUENCING DETERMINATION OF SODIUM IN PLANT MATERIAL\*

By W. M. BROADFOOT and G. M. BROWNING† (West Virginia  
Agricultural Experiment Station, Morgantown, W. Va.)

Study of some of the factors that influence the acid-base balance of plant material, such as differential application of fertilizer material, reaction of the soil, and maturity of the plant, was started in 1933. When the methods proposed by Frear (13) and Pierre (22) were used there appeared to be losses of certain elements during ignition. In order to determine the extent of such losses, an analysis for individual cations and anions was attempted.

For the determination of sodium the method of Barber and Kolthoff (2) was used at first, but difficulties were soon encountered. Other investigators, working largely with biological material, proposed various modifications of the original Barber-Kolthoff method. Butler (5) reviewed the literature on the determination of sodium and decided that the magnesium uranyl acetate method proposed by Caley and Foulk (8) should be applicable to plant material. Later the A.O.A.C. (9) suggested a slight modification of this procedure, which was found satisfactory for most plant materials. However, when 5–10 gram samples low in sodium are used, the amount of potassium present will often give high results, even though 100 ml. of the precipitating reagent is used. Consequently, a study of the various factors affecting the determination of sodium in plant materials was undertaken, and it is the purpose of this paper to present the results of this investigation and to propose a modified procedure that the writers found would eliminate some of the inaccuracies encountered.

#### REVIEW OF LITERATURE AND DISCUSSION

##### 1. *Removal of Sodium*

(a) *Acid extraction of ignited plant material.*—The A.O.A.C. procedure for determining sodium recommends moistening the plant sample with sulfuric acid before ignition. The results of a study of losses of sodium from sodium chloride ignited at various temperatures with and without sulfuric acid are shown in Table 1. It is evident that there is a definite loss of sodium at 560° C., even when sulfuric acid was added before ignition, but the loss is less than if no sulfuric acid were added. At temperatures of 510° C. or below, there was no loss of sodium. Similar results were obtained with plant samples. However, because sulfuric acid does de-

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crease loss of sodium and because in the early stages of ignition the temperature in the sample appears higher than it does in the muffle furnace, the use of sulfuric acid seems desirable as a precautionary measure against the loss of sodium.

From a large number of determinations it was found that ignition for 3-4 hours at 450° C. or for 2 hours at 510° C. would usually produce an ash free of carbon. If the containers are small a longer period of ignition is

TABLE 1.—*Recovery of Na from NaCl solution when ignited at different temperatures, with and without H<sub>2</sub>SO<sub>4</sub>*  
(Average of two or more determinations)

SAMPLE	Na ADDED	IGNITION REAGENT	IGNITION TEMPERATURE	Na RECOVERED	RECOVERY
	mg.		°C.	mg.	per cent
1	1.00	None	95*	1.012	101.2
2	1.00	None	560	.915	91.5
3	1.00	H <sub>2</sub> SO <sub>4</sub>	560	.951	95.1
4	1.00	None	510	.995	99.5
5	1.00	H <sub>2</sub> SO <sub>4</sub>	510	1.003	100.3
6	1.00	None	450	.990	99.0
7	1.00	H <sub>2</sub> SO <sub>4</sub>	450	1.011	101.1
8	1.00	None	400	.996	99.6
9	1.00	H <sub>2</sub> SO <sub>4</sub>	400	.992	99.2

\* Evaporated to dryness on hot plate; all other samples were evaporated to dryness on hot plate and then ignited in muffle furnace for 2 hours.

necessary. In any case it was found that moistening the sample with water after an initial ignition of an hour aids materially in obtaining a carbon-free ash. For routine work it is often convenient and satisfactory to leave the samples in the muffle overnight.

To determine whether glassware and silica could be used without danger of contamination, the writers added 10 ml. of sulfuric acid (1+10) to platinum, silica, and glass containers. The acid was evaporated on the hot plate and ignited for 30 minutes at 510° C., and sodium determinations were made in the usual manner. When silica and platinum crucibles or well-cleaned unetched 100 ml. Pyrex beakers were used, no precipitate was found, but when the beaker was badly etched, a precipitate formed that was equivalent to approximately 0.25 mg. of sodium. The beakers that were more than slightly etched gave a much smaller but still appreciable quantity of precipitate.

(b) *Acid extraction without igniting plant material.*—Terlikowski and Sozanski (24) extracted dried plant material with normal hydrochloric acid and found that the sodium content compared favorably with that obtained by ashing in the usual manner. Plant material extracted with hydrochloric acid sometimes gives a solution highly colored with colloidal organic material that must be removed if good results are to be obtained.

The authors were unsuccessful in securing a completely colorless extract, using either hydrogen peroxide or carbon black. It was necessary to evaporate the solution and ignite the residue in order to destroy the colloidal organic matter. Hence it appears that the hydrochloric acid extraction procedure offers no advantage over ashing the sample at the outset and for routine determinations is even more time-consuming.

## 2. Effect of Foreign Salts

(a) *Phosphorus*.—Phosphorus interferes with the determination, forming a co-precipitate of uranium phosphate with the sodium uranyl zinc acetate. Since an excess of salts interferes with the sodium determination and an appreciable amount of salts is present in the plant material, it is essential to keep the quantity of reagent at a minimum. Barber and Kolthoff (3) recommended the removal of phosphorus with magnesia mixture. Other investigators have used powdered zinc carbonate (21), uranium acetate (3, 4), powdered calcium hydroxide (4), zinc acetate (17), calcium chloride, ammonium hydroxide (9), and barium hydroxide (23). Koenig (16) found that the quantity of  $P_2O_5$  found in feldspar did not interfere when precipitation was carried out in the acid solution described in his procedure. An excess of calcium hydroxide was found to give quantitative removal of phosphorus, and solubility on prolonged washing with either cold or warm water was insignificant. Calcium chloride and ammonium hydroxide were found to reduce the concentration of phosphorus to the point where it did not interfere, but on washing an appreciable amount of the precipitate was soluble.

Inasmuch as sodium salts are very soluble in water it seemed that it might be possible to precipitate the phosphorus by adding before ignition a reagent such as the acetates of zinc, iron, barium, or calcium. These acetates would form insoluble oxides on ignition and would not increase the salt content when the sodium in the ash was dissolved in water. This procedure proved unsuccessful because (1) removal of the phosphorus was not quantitative, (2) large quantities of water were required to dissolve all the sodium, and (3) some of the materials were difficult to filter.

(b) *Potassium*.—Investigators (3, 6, 16) differ as to the quantity of potassium that interferes with the determination of sodium as sodium uranyl zinc acetate. When approximately 20 times more potassium than sodium is present it produces no deleterious effect. The results of Caley and Foulk (8) are of interest in this connection. Using magnesium uranyl acetate as the precipitating reagent, they found that when 10 or 20 mg. of sodium was precipitated in either 100 or 200 ml. of reagent, 250 mg. of potassium did not interfere. With 5 mg. of sodium and 50 ml. of reagent, satisfactory results were obtained in the presence of 100 mg. of potassium, but results were high when 200 mg. of potassium was present. Caley (6) found that when 0.2 and 0.5 mg. of sodium were precipitated with 100 ml. of magne-

sium uranyl acetate in the presence of 100 mg. of potassium, the results were about 95 and 15 per cent high, respectively. The data therefore show that the amount of potassium that causes interference is dependent upon the amount of sodium present. With plant material, potassium is generally high with respect to sodium and will frequently cause interference. The effect of potassium on the precipitation of sodium from a standard sodium chloride solution, as found in this laboratory, is shown in Table 2.

TABLE 2.—*Effect of K concentration on precipitation of Na from a standard NaCl solution*

(Average of two or more determinations)

SAMPLE	PRECIPITATING REAGENT	K ADDED	Na ADDED	WEIGHT Na*	RECOVERY
	ml.	mg.	mg.	mg.	per cent
1	10	None	1.00	.998	99.7
2	10	10	1.00	1.007	100.7
3	10	20	1.00	1.005	100.5
4	10	50	1.00	1.190	119.0
5	25	80	0.50	1.595	319.0
6	50	80	0.50	.501	100.2
7	100	80	0.50	.494	98.8
8	100	200	0.50	.875	175.0
9	50	125	1.00	4.860	486.0
10	100	125	1.00	1.002	100.2

\* Calculated from the weight of precipitate found.

It is to be seen from these data that when 10 ml. of reagent and 1.0 mg. of sodium were used, 20 mg. and 50 mg. of potassium gave about 5 and 19 per cent high results, respectively. With large quantities of sodium the precipitation of a small quantity of potassium does not significantly affect the results, but with small quantities of sodium, which is generally the case with plant material, an appreciable error will result from the precipitation of small quantities of potassium. Therefore it appears that the concentration of potassium is more important than the ratio of potassium to sodium.

When potassium is present in sufficient quantities to cause interference (approximately 15 mg. of potassium per 10 ml. of precipitating reagent), it is possible to remove it with ammonium perchlorate. This procedure requires care and time if satisfactory results are to be obtained.

Nydahl (19) recommended double precipitation as a means of eliminating the interference of potassium because above a certain concentration some of the potassium is precipitated along with all the sodium. When the precipitate that contains a small proportion of the original potassium is dissolved and precipitated, the potassium concentration has been reduced to the place where it no longer interferes. With 50 mg. of potassium, 1.0

mg. of sodium, and 10 ml. of reagent the results were about 10 per cent high (Table 2). Dissolving the precipitate in 2 ml. of water and again precipitating the sodium in the usual manner gave theoretical recovery. When the amount of potassium was increased to 125 mg. the amount of precipitate was approximately twelve times the theoretical value. Double precipitation materially decreased the amount but not to the theoretical value. It appears, therefore, that with high concentration of potassium in small volumes of reagent sufficient potassium is carried down either in the form of precipitate or by occlusion to cause interference. Furthermore, it has been shown that the formation of excessive amounts of precipitate will desaturate the reagent to the place where solubility of the precipitate becomes a factor. For certain types of solution double precipitation probably can be used to advantage. However, when the plant ash is taken up in acid, salts will usually start coming out at about 5.0 ml. This volume requires at least 25 ml. of reagent to maintain a 1 to 5 sample solution-reagent ratio, and with this dilution the interference of potassium in many cases will be eliminated. As will be shown later in this paper it is possible to eliminate potassium interference by precipitating in nitric acid solution. Therefore, there is no particular advantage in double precipitation when working with plant material.

If concentration of potassium is the important factor, then it should be possible to increase the amount of precipitating reagent and eliminate the need for removing the potassium. The data in the literature recommend a sample solution ratio of 1 to 20 when magnesium uranyl acetate reagent is used. Salts start coming out at about 5.0 ml. from acid extraction of 5.0 grams of the ashed plant sample. According to the A.O.A.C. procedure (18), 100 ml. of reagent is necessary if the above sample solution-reagent ratio is to be maintained. The data in Table 2 show that when 100 ml. of uranyl zinc acetate reagent is used, 125 mg. of potassium (approximately the amount found in 5.0 grams of the average plant material) will not interfere. However, it is not uncommon to find plants containing up to 200 mg. of potassium (4.0% K) and some have been reported (11) as containing as much as 400 mg. of potassium in a 5.0 gram sample. Under these conditions, unless the quantity of reagent is increased accordingly, high results are to be expected.

(c) *Other elements*—The quantity of other elements such as calcium, strontium, magnesium, barium, and lithium found in plant material would not be expected to interfere according to the data of Barber and Kolthoff (3), Caley and Foulk (8), and others (16, 19).

### 3. *Effect of Temperature*

Caley and Foulk (8), Dobbins and Byrd (12), and Barber and Kolthoff (3) found that low results were obtained when temperatures during precipitation were above 20° C. On the other hand, Koenig (16) found that

up to and including 40° C., precipitation was complete in 30 minutes when the temperatures of precipitation and filtration were held constant. He further observed that an increase from 20° to 30° C. between precipitation and filtration did not affect the results, but a decrease in temperature of 30° to 20° C. from precipitation to filtration resulted in an appreciable error. Although a detailed investigation of the effect of temperature was not made in this study, satisfactory results were obtained throughout the year with temperatures ranging from 18° to 35° C.

#### 4. *Ratio of Sample Solution to Reagent*

Chen (10), working with alcoholic solutions containing 0.04–0.2 mg. of sodium, used a sample solution-reagent volume of 1 to 3. Other investigators have insisted on a ratio of 1 to 10 or even as high as 1 to 20. Koenig (16), using amounts of sodium commonly found in feldspar with a sample volume of 5 ml. and precipitation period of 30 minutes at room temperature, found that sample solution-reagent ratios in excess of 1 to 4 gave good results regardless of the excess up to and including a ratio of 1 to 25. Since the reagent is relatively expensive, there is an advantage in using the minimum quantity of reagent necessary to give satisfactory results. In this study, when working with sodium chloride solutions, the writers found that sample solution-reagent ratios including and in excess of 1 to 5 gave good results, and that with plant material, if the volume of precipitating reagent was increased to eliminate the effect of potassium, there was a sample solution-reagent ratio of 1 to 5 or even 1 to 10.

#### 5. *Effect of Acidity of Sample Solution*

Most of the previous work has been done with aqueous solutions. Koenig (16) studied the effect of varying the acidity of the sample solution upon the accuracy of the method. He found that the amount of perchloric acid should be held below 1 ml. per 5 ml. volume, though higher concentration did not show a large deviation from the true value. In Table 3 is shown the recovery of sodium when concentrated nitric acid was used to increase the acidity. A solution-reagent ratio of 1 to 10 was maintained in all cases.

From these data it is evident that amounts up to and including 1.50 ml. of concentrated nitric acid did not interfere with the recovery of sodium, but that only a trace of precipitate formed when 2.0 ml. of acid was used.

Koenig (16) observed that phosphorus in amounts found in feldspar, when added to phosphorus-free samples of feldspar, did not interfere if the solution was made acid with perchloric acid. Likewise, approximately 1.0 mg. of phosphorus as sodium acid phosphate did not significantly affect the data. It seemed, therefore, that it might be possible to use concentrated nitric acid and prevent the phosphorus in plant material from

TABLE 3.—*Effect of varying quantity of  $\text{HNO}_3$  on recovery of Na from a standard solution of NaCl*  
(Average of two or more determinations)

SAMPLE	CONC. $\text{HNO}_3$	Na ADDED	Na FOUND	RECOVERY
	ml.	mg.	mg.	per cent
1	None	1.0	1.010	101.0
2	.50	1.0	1.012	101.2
3	1.00	1.0	1.018	101.8
4	1.25	1.0	.990	99.0
5	1.50	1.0	.992	99.2
6	2.00	1.0	trace	—

interfering even though the amount may be several times larger than that found in feldspar. The use of the amount of phosphorus found in the average plant material and 1.5 ml. of concentrated nitric acid per 10 ml. of reagent produced a bulky precipitate, indicating that concentrated nitric acid will not prevent interference of the amount of phosphorus found in plant material. Likewise, 70 per cent perchloric acid used in the amounts that would not hinder the formation of triple acetate proved ineffective in preventing the precipitation of uranium phosphate.

The acidity of the solution as a means of reducing potassium interference in the determination of sodium from a standard sodium chloride solution is shown in Table 4. In the absence of acid the amounts of potassium commonly found in plant material definitely give high results. Increasing the acidity of the solution decreases the amount of precipitate until the error is small with 1.5 ml. of concentrated nitric acid. The slightly high results may be due in part to the presence of a small amount of sodium in the commercial potassium salts. It is also possible that the acid dissolved some of the precipitate rather than preventing the precipitation of the potassium along with the sodium. However, the fact that sodium alone precipitates quantitatively as sodium uranyl zinc acetate in solutions containing 1.5 ml. of concentrated nitric acid, indicates that the effect of increasing the acidity is to prevent the precipitation of the potassium. The data show that if the acidity is carefully controlled the interference of potassium can be largely eliminated.

#### 6. Size of the Plant Sample

It is not uncommon to find plants that contain as little as 0.002 per cent of sodium and as much as 4 per cent of potassium. Assuming a sample with this composition, a 10 gram sample would contain 0.2 mg. of sodium and 400 mg. of potassium. One mg. of sodium yields 66.8 mg. of precipitate and is a very convenient amount to work with. Satisfactory results can also be obtained with 0.5 mg., and even 0.2 mg. can be determined with considerable precision. An average error of 7 per cent was obtained from

a series of eight determinations on an individual plant sample containing about 0.2 mg. of sodium. It is evident from Table 2 that about 250 ml. of reagent would be required to prevent 400 mg. of potassium from interfering. Data on the individual elements in plant material are usually reported to 2 decimal places, while the acid-base balance is expressed in milliequivalents per 100 grams of sample. If some value for sodium could be decided upon, below which the results would be considered as a trace, then the size of sample could be limited. Assuming that a plant contains 0.01 per cent of sodium, then a 2.0 gram sample would contain 0.2 mg. of sodium or 0.43 m.e. per 100 grams. A 7 per cent error of determination on this small amount of sodium represents about 0.2 m.e. per 100 grams. When it is considered that differences of 0.02 per cent are readily found between duplicate determinations of calcium, potassium, and the other elements, and that these differences may represent as much as 0.5 m.e. per 100 gram sample, it may be seen that the error from the sodium determination is indeed small. In fact, the sodium could be entirely omitted from the final calculation, and the acid-base balance would be within experimental error. Some studies may require the estimation of sodium less than 0.01 per cent. However, in general, if samples contain less than 0.01 per cent sodium, it can be considered as insignificant. By limiting the size of sample to 2.0 grams, only 80 mg. of potassium would be present if the sample contained 4 per cent potassium. The average plant will generally contain less than half this amount; therefore the possibility of interference has been materially reduced. Consequently, the volume of reagent required can be decreased.

### *7. Methods of Recovering the Precipitate*

The sodium uranyl zinc acetate precipitate is usually collected in weighed Gooch crucibles charged with asbestos. The centrifuge has been used to advantage in many of the micro methods of analysis, including the method for sodium. Using graduated 15 ml. Pyrex centrifuge tubes that had been previously weighed, the writers made a series of determinations on a standard solution of sodium chloride. The sodium was precipitated in the tubes; 10 ml. of the precipitating reagent was added to 1 or 2 ml. of sample solution, stirred 1 minute, and allowed to stand at room temperature at least 15 minutes before being centrifuged for 5 minutes at 2000 r.p.m. The liquid was carefully poured off, and the tubes were allowed to drain by inverting on a soft cloth. The precipitate was washed with 5 ml. of alcoholic wash solution by breaking up the precipitate with a glass rod and recentrifuging as described above. The washing procedure was repeated with ether. Finally the precipitate was dried for 15 minutes in an oven at 105°–110° C. and weighed. This procedure was found to be particularly effective when potassium was present in the sample solution in amounts less than 20 mg. Even with 50 mg. of potassium it is possible,

by double precipitation or by increasing the acidity (Table 4) to obtain theoretical recovery of the sodium; 50 ml. centrifuge tubes were also used if the volume of sample solution was more than 2.0 ml. or when the concentration of potassium necessitated increasing the volume of reagent. The error in weighing is greater when 50 ml. tubes are used, and with small amounts of sodium (0.2 mg.) the results are not satisfactory.

Titration and colorimetric methods have been proposed (1, 12, 14, 15, 25, 26) for estimating the amount of sodium in the sodium uranyl zinc

TABLE 4.—*Acidity of solution as a means of reducing the interference of K in determination of Na*  
(Average of two or more determinations)

SAMPLE	CONC. HNO <sub>3</sub>	K ADDED	Na ADDED	Na FOUND	RECOVERY
	ml.	mg.	mg.	mg.	per cent
1	None	None	1.0	1.020	102.0
2	1.00	None	1.0	1.018	101.8
3	1.50	None	1.0	0.992	99.2
4	None	62.5	1.0	5.790	579.0
5	1.5	62.5	1.0	1.008	100.8
5	None	125.0	1.0	11.500	1150.0
7	1.00	125.0	1.0	4.320	432.0
8	1.25	125.0	1.0	2.190	219.0
9	1.50	125.0	1.0	1.075	107.5

acetate precipitate. The titration procedures described by Ball and Sadusk (1), Holmes and Kirk (15), and Dobbins and Byrd (12) were tried in this laboratory and difficulties were encountered. However, after the procedures had been standardized satisfactory results were obtained, but for routine determinations the gravimetric procedure was found to be more desirable than the titration methods studied.

To decrease the effect of salt concentration, the interference from potassium, and the need for evaporating the solution, the following series of determinations with aliquots was carried out: 10 ml. of 0.50 *N* hydrochloric acid was added to the plant ash and allowed to react. With phenolphthalein as an indicator, an excess of powdered calcium hydroxide was then added to precipitate the phosphorus, and the solution was filtered through a small dry filter paper; 2.0 ml. and 5.0 ml. portions of the filtrate were then placed in 15 ml. and 50 ml. centrifuge tubes, respectively, and the sodium was precipitated and weighed in the manner described above. Following this procedure, determinations of sodium were made on a standard sodium chloride solution, a sample of cabbage alone, and cabbage to which a known amount of sodium had been added. It was found that satisfactory recovery can be expected when the aliquots contain as much as 0.25 mg. of sodium. Most of the common plant materials studied were much lower in sodium than this particular sample of cabbage (0.9% Na).



Consequently this procedure is not suitable for the determination of sodium in all plant material.

### 8. *Washing the Sodium Uranyl Zinc Acetate Precipitate*

Alcohol, acetone, and glacial acetic acid, saturated with the triple salt, have been recommended as wash liquids. As pointed out by Hoffman and Osgood (14), each of these reagents produces a precipitate when brought into contact with uranyl zinc acetate that may represent an appreciable error, especially when small quantities of sodium are being used. They observed that the amount of precipitate is greatest with alcohol, is much smaller with 15 per cent acetic acid in alcohol, and is almost negligible in acetic acid. Washing the precipitate with glacial acetic acid saturated with the triple salt has given consistently low results in this laboratory. On the other hand, alcohol saturated with the triple salt by shaking over a period of 30 minutes at room temperature was found to give the correct value. However, the same alcohol, when saturated with the triple salt and allowed to stand for several days, formed a precipitate on the walls of the container, and washing the triple salt with this solution gave low results, indicating that it was unsaturated. Therefore it is advisable, regardless of which wash solution is used, to check frequently on known standards to determine whether theoretical recovery is being obtained.

#### METHOD

##### REAGENTS

(a) *Uranyl zinc acetate*.—Prepare as directed by Koenig (16) except to add 15 ml. of  $\text{HNO}_3$  per 100 ml. of reagent.

<i>Solution A</i>		<i>Solution B</i>	
$\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$	365 grams	$\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$	1095 grams
Glacial acetic acid	205 ml.	Glacial acetic acid	34 ml.
Water	1790 ml.	Water	1242 ml.

Bring the uranium and zinc acetate into solution in water by gentle warming and stirring. (Solution A will remain cloudy because of the hydrolysis of the uranium salt.) When the acetates are in solution, add 205 ml. of glacial acetic acid to Solution A, and 34 ml. to Solution B. Mix the solutions while hot, allow to cool to room temperature, and finally treat with ca. 0.2 gram of sodium uranyl zinc acetate to insure saturation. Add to the mixture 15 ml. of  $\text{HNO}_3$  per 100 ml. of reagent, allow to stand 24 hours, and filter before using. (This method of preparing the reagent is more rapid than when the acetic acid is added at the beginning.)

(b) *Saturated ethyl alcohol*.—Saturate ethyl alcohol (95%) with the triple salt by shaking at room temperature for 30 minutes and filtering before using. (The solution should be prepared fresh every few days because it becomes desaturated and low results follow.)

#### PROCEDURE

To 2.0 grams of plant material in a platinum crucible add 5.0 ml. of  $\text{H}_2\text{SO}_4$  (1+10) and water if necessary to moisten the sample. Heat on a hot plate to expel the excess water and ignite for 5 hours at  $510^\circ\text{--}540^\circ\text{C}$ . Add to the ash 1.0 ml. of  $\text{HCl}$  and 15–25 ml. of water and heat on the hot plate for 30 minutes. Precipitate the

phosphorus by adding a slight excess of powdered  $\text{Ca}(\text{OH})_2$ , using phenolphthalein as an indicator. Filter into a 100 ml. beaker and evaporate to 5.0 ml. or less if no salts separate. Cool, add 30 ml. of the uranyl zinc acetate and stir vigorously for 1-2 minutes. If the amount of sodium is extremely low (less than 0.2 mg.), precipitation is slow and may require considerable stirring. Allow the mixture to stand 30 minutes, and filter with suction through a weighed Gooch crucible charged with asbestos that has been previously washed consecutively with alcohol and ether (C. P. anhydrous) and heated for 15 minutes in an oven at  $105^\circ\text{C}$ . Remove the last traces of triple salt from the beakers with small portions of the reagent (ca. 2 ml.). Wash the precipitate with four 2.0 ml. portions of 95% alcohol saturated with the triple salt and finally with a 5.0 ml. portion of the ether. Dry in oven at  $105^\circ\text{--}110^\circ\text{C}$ . for 15 minutes, cool, and weigh. Weight of sodium uranyl zinc acetate precipitate  $\times 0.01495$  = weight of sodium.

### SUMMARY

Some factors affecting the determination of sodium in plant material have been discussed and a modified procedure of the Barber and Kolthoff method presented.

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MODIFICATION OF THE ASSAY OF OINTMENT  
OF MERCURIC NITRATEBy R. K. SNYDER (American Pharmaceutical Association,  
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In a project under way in the Laboratory of the American Pharmaceutical Association, it was necessary to provide a method of analysis for ointment of mercuric nitrate, which is official in National Formulary VI. Various procedures were investigated, and it was found that the method listed as tentative in *Methods of Analysis*, A.O.A.C., 1940, page 622, held the most promise. However, the extended period of heating with potassium permanganate and the difficulty in determining when oxidation was complete made it doubtful whether this part of the method would be generally acceptable. It was therefore considered necessary only to provide another method of oxidation, since the first part of the A.O.A.C. assay involving the extraction of the mercuric nitrate from the ointment base was found to be satisfactory.

## EXPERIMENTAL WORK

The details of the method as finally worked out are as follows:

Transfer an accurately weighed sample of the ointment, 3-5 grams, to a Kjeldahl flask and add 40 ml. of  $\text{HNO}_3$  (1+1). Place a small funnel in the neck of the flask and heat for 1½ hours, boiling just enough to maintain agitation. Cool under running water, swirling the flask to prevent the undissolved matter from solidifying in one large piece. Dilute to 200 ml. and filter through an unwetted filter. Transfer 100 ml. of the filtrate to a Kjeldahl flask, add 10 ml. of  $\text{H}_2\text{SO}_4$ , and heat until white fumes of  $\text{SO}_2$  are visible. Immediately remove the flame and allow to cool. Add 5 ml. of  $\text{HNO}_3$  and heat again at a medium rate until the rate of boiling decreases markedly. Cool, dilute to 100 ml., and cool again if necessary. Titrate with 0.1 *N*  $\text{NH}_4\text{SCN}$ , using  $\text{FeNH}_4(\text{SO}_4)_2$  as the indicator. Each ml. of 0.1 *N*  $\text{NH}_4\text{SCN}$  = 0.01003 gram of Hg.

The results obtained by this method on an ointment made in the Laboratory are as follows:

Sample	Mercury per cent
1	7.9
2	7.7
3	7.6

It should be noted that any prolonged heating of the mercury in the presence of sulfuric acid only is avoided in this procedure. The digestion with nitric acid is not carried to the point where white fumes are visible as it is preferable to retain some nitric acid. No appreciable mercury is lost, as was shown by carrying out the determination in a distillation apparatus equipped with glass joints. The distillates required only one drop of ammonium thiocyanate for a weak end point and two for a strong one.

This method warrants further study by collaborative workers because of its ease and simplicity.

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## DETERMINATION OF STARCH IN SAUSAGE AND OTHER MEAT PRODUCTS

By WARREN C. McVEY (U. S. Department of Agriculture,  
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In the preparation of many kinds of chopped or comminuted meat products it is customary to add cereal or flour prepared from starchy grain. Under the Meat Inspection regulations of the U. S. Department of Agriculture, the addition of cereal or starchy vegetable flour to sausage is permitted under appropriate label declaration in quantities up to 3.5 per cent of the finished product. The use of cereal is also permitted in appropriate quantities as an ingredient in such other products as meat loaf, scrapple, pâté, and potted meat.

All the methods found in the literature that have been used or proposed for the detection or determination of cereal in meat products are based on a determination of starch, which is the principal constituent of most cereals. The proportion of starch in a cereal varies somewhat with the grain from which it is made and its method of processing, but it is quite uniform for flours or cereals of the same type from each variety of grain. Since only a few types of cereals have been used as constituents of meat products, a determination of the starch content of a meat product may be used as a measure of the quantity of cereal added.

One of the earliest quantitative methods used for the determination of starch in meat products was a modification by Bigelow (1) of the classical Mayrhofer procedure, in which the meat product is digested with 8 per cent alcoholic potassium hydroxide solution until the protein and fatty material have disintegrated. After dilution with alcohol, the starchy residue is collected on a filter and washed, after which it is dissolved in a dilute solution of aqueous potassium hydroxide. After filtration, the starch is precipitated from an aliquot of the solution with alcohol, filtered off, dried, and weighed. This procedure was found impracticable because of the difficulty in filtrations, and was abandoned in favor of the Price modification of the same method (2) (3), which differs from the Bigelow procedure in that the starchy residue is hydrolyzed with hot dilute sulfuric acid, and the quantity of starch is calculated from a determination of reducing sugars. The Price modification is the method in use at the present time by the Association of Official Agricultural Chemists (4), and while it produces quite acceptable results in the hands of an experienced analyst, it is open to the objections that it is somewhat tedious and involved, requires a carefully developed technic on the part of the analyst, and often yields results that are too high, owing to the conversion to dextrose of

some of the hemicellulose and fibrous material. This last shortcoming has become especially significant in recent years because of the increasing popularity of soybean preparations as ingredients of certain meat products.

Among other methods that have been proposed for the determination of cereal in meat products is the iodine precipitation method of Edwards, Nanji, *et al.* (5) (6), which involves the digestion of the meat product with alcoholic potassium hydroxide, the gelatinization of the starchy residue with dilute aqueous potassium hydroxide, and the precipitation of the starch by means of iodine and a coagulating agent such as alcohol. This procedure gives fairly consistent results although the filtrations are often difficult. It is also open to the objection that the starch-iodine complex is unstable and of somewhat uncertain composition.

In an effort to develop a simpler and more direct method for the determination of starch in meat products in the presence of hemicelluloses, the method of Rask (7) (8) for the determination of starch in cereals was investigated. In this procedure the starchy material is treated with approximately 21 per cent hydrochloric acid at room temperature, and after filtration the starch is precipitated with alcohol, as in the Bigelow method. A basically similar procedure by Grossfeld (9) for the determination of starch in sausage by a measurement of the optical activity of the starch solution was also investigated. After a number of modifications of the Rask procedure had been tried in an effort to make the filtrations less tedious, a procedure was developed by which the starch content of a meat product can be determined more rapidly than by the present tentative A.O.A.C. method (4), and which is accurate in the presence of materials containing large quantities of hemicellulose.

The following tables show the recovery of starch added to a sausage product in the form of corn flour, and a comparison of the recovery of added starch by the proposed method and by the A.O.A.C. method, both in the presence and absence of soybean flour.

#### PROCEDURE

Using a balance sensitive to 0.1 gram, weigh 10 grams of the comminuted and mixed meat product into a 150 ml. beaker. Add 50 ml. of an 8% solution of KOH in 95% alcohol, break up lumps with a stirring rod, and heat on the steam bath for 30–45 minutes, or until the product has been completely disintegrated. Dilute with 40–50 ml. of 95% alcohol, and add, with stirring, ca. 1 gram of filter-cel, of a grade unaffected either by dilute HCl or dilute KOH solution. After a few minutes, filter with the aid of suction through a Gooch crucible fitted with a disk of filter paper, wash thoroughly with 95% alcohol, and suck as dry as possible. Holding the crucible and contents inverted over the same beaker used for the digestion, dislodge the contents by tapping the sides of the crucible. Remove any material remaining in the crucible by means of a small piece of filter paper, and add the paper to the material in the beaker.

Add 5.7 *N* HCl in small portions to the cereal-filter-cel mixture in the beaker while stirring or kneading the mass with a stirring rod until the contents assume a

TABLE 1.—*Recovery by the proposed method of cereal added to sausage*

CEREAL ADDED	STARCH RECOVERED	RATIO: % CEREAL ADDED % STARCH RECOVERED	CEREAL RECOVERED (% STARCH×1.27)	DIFFERENCE
<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
0.0	0.0	—	—	—
0.0	0.12	—	—	—
0.5	0.40	1.25	0.51	+ .01
1.0	0.82	1.22	1.04	+ .04
1.0	0.80	1.25	1.02	+ .02
1.5	1.24	1.21	1.57	+ .07
1.5	1.18	1.27	1.50	.00
2.0	1.64	1.22	2.08	+ .08
2.0	1.60	1.25	2.03	+ .03
2.0	1.54	1.30	1.95	— .05
2.5	1.96	1.27	2.49	— .01
2.5	1.96	1.27	2.49	— .01
3.0	2.30	1.30	2.92	— .08
3.0	2.34	1.28	2.97	— .03
3.5	2.78	1.26	3.53	+ .03
3.5	2.76	1.27	3.50	.00
3.5	2.80	1.25	3.56	+ .06
4.0	3.16	1.26	4.02	+ .02
4.0	3.14	1.27	3.98	— .02
4.0	3.16	1.26	4.02	+ .02
4.5	3.50	1.29	4.44	— .06
4.5	3.62	1.24	4.59	+ .09
4.5	3.60	1.25	4.57	+ .07
5.0	3.92	1.27	4.98	— .02

TABLE 2.—*Effect of soybean flour on the recovery of starch from sausage by the proposed method and by the A.O.A.C. method*

STARCH ADDED	SOYBEAN FLOUR ADDED	STARCH RECOVERED— BY PROPOSED METHOD	BY A.O.A.C. METHOD
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.0	0.0	0.0	0.18
0.0	1.0	0.0	0.46
1.0	0.0	0.96	1.18
1.0	1.0	0.98	1.40
2.36	0.0	2.32	2.39
2.36	2.0	2.36	2.76

creamy consistency. Transfer the contents, together with the fragments of filter paper, quantitatively to a 100 ml. volumetric flask with the aid of a jet of 5.7 *N* HCl from a small wash bottle, and add more of the HCl if necessary to make a volume of 50–60 ml. in the flask. Stopper the flask, shake vigorously for 3–5 minutes, and let stand about 15 minutes longer with occasional shaking; or, preferably, shake continuously for 10 minutes in a mechanical shaker. Make up to volume with water, mix thoroughly, and filter with the aid of suction through a layer of asbestos in a Gooch crucible.

In another Gooch crucible prepare a moderately thin asbestos mat, fill the crucible ca. two-thirds full of filter-cel, dry at 100°C., and when cool tare on an analytical balance. By means of a graduate measure 115 ml. of 95 % alcohol into a 250 ml. beaker. Pipet a 50 ml. aliquot of the filtered starch solution into the alcohol and stir vigorously. Then before the precipitated starch has coagulated or settled, pour the filter-cel from the tared crucible into the alcohol-starch mixture and again stir thoroughly. Allow to settle for ca. 5 minutes, then using suction filter through the tared Gooch crucible and wash thoroughly with 95 % alcohol, using a rubber-tipped stirring rod, if necessary, to dislodge all starchy material from the sides of the beaker. Dry overnight at 100°C., or for 1½ hours at 125°C., cool in a desiccator, and weigh immediately on removal from the desiccator, as the material is hygroscopic.

Multiply the weight of starch found by 20 to obtain the percentage of starch in the sample. To obtain the percentage of cereal, multiply the starch (per cent) found by the appropriate factor (the reciprocal of the proportion of starch in the particular cereal product used), which in the case of corn flour is 1.27.

#### SUMMARY

A procedure for the determination of starch in sausage and other meat products is proposed. The residue remaining after digestion of the meat product with alcoholic potassium hydroxide is treated with hydrochloric acid, which dissolves the starch. After filtration the starch is precipitated by alcohol, and with the aid of filter-cel is collected in a Gooch crucible, dried, and weighed. The proposed procedure is more rapid and convenient than the present tentative A.O.A.C. method, and it is reliable in the presence of hemicellulose material.

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RELATION OF QUANTITY OF MINERAL ELEMENTS IN  
ASH TO ASHING CONDITIONS IN PLANT MATERIAL

By J. L. St. JOHN and M. C. MIDGLEY\* (Division of Chemistry,  
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The object of a method for the determination of ash in feeds or in plant material is to produce a result that gives the closest possible correlation between the percentage of ash reported and the sum of the various mineral elements actually present in the sample. No evidence that any such ashing procedure has been published was found by the writers. Therefore, studies of this relation were made in this laboratory during the early months of 1940. The more recently developed methods for the oxidation of the organic matter by perchloric acid were utilized when they were applicable; nitric-perchloric acid digestion was used in a determination of the "total" quantity of calcium, phosphorus, sulfur, and potassium; and the sodium-carbonate fusion method (1) was utilized for the determination of total chlorine.

Comparisons were made on two feed samples similar to those described by St. John (2). These were ashed at temperatures of 500°, 600°, and 700°C., respectively; each of the five elements mentioned previously was determined on the resulting ash; and the results were compared with the data obtained by the methods designed to determine the total amount of these elements in the feed. The methods utilized for the determination of the different elements after ashing were those given in *Methods of Analysis, A.O.A.C.*, 1935 (1). In all cases except one the ashing was done by the 1940 official method (3). The temperatures used were 500°, 600°, and 700°C. This procedure gives a direct comparison of the quantity of different minerals in the ash prepared at these three temperatures with the total amount obtained by the more recent digestion methods for bringing mineral elements into solution.

The perchloric acid digestion procedure utilized was very similar to that described by Gerritz (4) and simultaneously by Giesekeing, Snyder and Getz (5); by Smith (6), Cook (7), and later by St. John. All these authors emphasized that caution is necessary during the perchloric acid digestion, although difficulty has not been experienced in this laboratory. The results obtained from the above plan and methods are presented in Table 1, and are expressed in terms of percentage of the element in the original sample of feed rather than the percentage in the ash.

To compare further the recovery of the different elements in the ash, the ratios of amount recovered to the total in feed were calculated, and these ratios are presented in Table 2.

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\* The authors wish to express their appreciation to Dave Snoeberger and Russel Edwards for efficient assistance in the analytical work.



TABLE 1

FEED A				FEED B			
HClO <sub>4</sub>	500°	600°	700°	HClO <sub>4</sub>	500°	600°	700°
<i>Calcium</i>							
0.95	0.95		0.97				
0.97	0.95		0.97				
0.95	0.95		0.97				
1.00	.98	1.00	.99	.23	.24	.24	.26
.98	.97	.98	.97	.24	.23	.23	.24
Av.	.97	.96	.99	.24	.24	.24	.25
<i>Phosphorus</i>							
.98	.93	.93	.92	.52	.52	.50	.51
.97	.91	.93	.92	.52	.50	.50	.49
Av.	.98	.92	.93	.52	.51	.50	.50
<i>Sulfur</i>							
0.18	0.052		0.033				
0.18	0.051		0.036				
0.18	0.054		0.035				
0.178	0.047	0.043	0.046	0.142	0.031	0.023	0.035
0.198	0.046	0.040	0.042	0.138	0.028	0.025	0.032
Av.	.183	.050	.042	.140	.030	.024	.034
<i>Chlorine</i>							
Fusion				Fusion			
1.00	0.89	0.88	0.79	0.84	0.65	0.64	0.51
1.00	0.87	0.88	0.78	0.85	0.66	0.63	0.52
Av.	1.00	.88	.78	.84	.66	.64	.51
<i>Potassium</i>							
0.767	.66	.69	.55	0.647	.60	.52	.47
0.750				0.655			
0.780				0.655			
0.766							
Av.	.766			.652			

The results in Tables 1 and 2 indicate certain definite trends. All the percentages in the case of calcium obtained by the digestion of the feed samples and by ashing these same samples at different temperatures give nearly identical results. The same amount of calcium is recovered in the

TABLE 2

	FEED A			FEED B		
	500°	600°	700°	500°	600°	700°
Calcium	0.990	1.020	1.000	1.00	1.00	1.04
Phosphorus	.947	.952	.942	.983	.967	.963
Sulfur	.273	.230	.208	.214	.171	.243
Chlorine	.88	.88	.78	.786	.762	.607
Potassium	.862	.901	.718	.920	.798	.721

ash at the three temperatures, and it is equal to the amount of calcium recovered by wet digestion with nitric-perchloric acid. Compared with the perchloric acid digestion, some phosphorus is lost during ashing, although the loss is about the same at different temperatures. Differences in temperatures and in ashing procedures have little effect on the quantities of calcium and phosphorus recovered, and such results are essentially identical with those obtained by the perchloric acid digestion. Thus, it appears that any of these ashing temperatures may be utilized to obtain relative quantities of calcium and phosphorus. However, the ratio of these quantities to the percentage of ash will vary, since the percentage of the ash itself changes with a variation in the temperature of ashing. Thus, any of these temperatures might be satisfactory from the standpoint of these two elements.

The results obtained for sulfur and chlorine present a different situation from that shown by calcium and phosphorus. Both samples show a much higher percentage of sulfur by perchloric acid digestion. The quantity of sulfur found in the ash at each temperature with both samples is much below the total amount in the feed. The temperature of ashing appears to influence to a small extent the quantity of sulfur recovered. Results at all three temperatures agree closely. The quantity of sulfur retained in the ash is one-fourth to one-fifth of the quantity shown by the perchloric acid digestion.

The data for chlorine show a still different situation. The loss of chlorine by ashing is not so large as in the case of sulfur, and the temperature of ashing influences the amount of chlorine remaining in the ash. Also the relative amount of chlorine in the ash varies between the two samples used. About 78 per cent of the chlorine is found in the ash of Feed B at 500° while Feed A at 500° retains about 88 per cent chlorine. The same type of variation exists at 700°, where 60 per cent of the chlorine remains in the ash of Feed B and 78 per cent in the ash of Feed A.

With potassium the temperature of ashing appears to influence the quantity recovered. Here, as in the case of chlorine, there is a greater loss of potassium when ashing is done at 700° than at 500° or 600°. The quantity of potassium at 500° or 600°C. is not markedly less than that obtained

by perchloric acid digestion, while there appears to be a material loss of potassium in the case of both feeds when the samples are ashed at a temperature of 700°C.

Since the percentage of ash shown in St. John's report (2) varied with temperature, the ratio of the percentage of each element as determined in the ash prepared at the three temperatures, in relation to the average per cent of ash in the feed as reported in 1940, was calculated, and these results appear in Table 3. The ratio for calcium shown in Table 3 increases with temperature owing to the decreasing percentage of ash. There is also a tendency for the phosphorus ratio to increase with temperature since the decrease in ash percentage is greater than the decrease in phosphorus recovery. An increase in ratio with increase in temperature is not evident in connection with the elements sulfur, chlorine, and potassium. The tendency may be in the opposite direction.

TABLE 3

	FEED A			FEED B		
	500°	600°	700°	500°	600°	700°
Calcium	.134	.145	.152	.039	.041	.046
Phosphorus	.129	.136	.144	.083	.083	.092
Sulfur	.007	.006	.006	.005	.004	.006
Chlorine	.123	.129	.122	.107	.108	.093
Potassium	.092	.101	.086	.098	.088	.086

More complete information regarding the relation of temperature of ashing to recovery of mineral elements should be obtained before the optimum or most desirable temperature of ashing is established. These added data should be correlated with those already reported and with further information regarding the appearance and the amount of "carbon" remaining in the ash. Further data of the type presented here may also emphasize the relation of ashing conditions to methods of determination of elements such as potassium when analytical methods are based on an analysis of the ash of plant or animal material.

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## DETERMINATION OF DIETHYLENEGLYCOL-MONOETHYL ETHER

By IRWIN S. SHUPE (Cosmetic Division, U. S. Food and Drug Administration, Federal Security Agency, Baltimore, Md.)

Diethyleneglycol-monoethyl ether [2-( $\beta$ ethoxy-ethoxy) ethanol] has been proposed for use in various types of cosmetics, including vanishing creams and lotions.<sup>1</sup> The commercial material is often referred to by the name "carbitol"<sup>2</sup> in the literature. It is described<sup>3</sup> as a colorless slightly hygroscopic liquid, boiling at 202°C., with specific gravity of 0.990 at 20/20 and refractive index of 1.4244[26/D].

A volumetric procedure is proposed for the determination of diethyleneglycol-monoethyl ether by an application of the xanthate reactions. The method is applicable to the relatively small quantities of the glycol ether that may be found in cosmetics.

### PREVIOUS WORK

Apparently no methods have been published for the quantitative determination of diethyleneglycol-monoethyl ether.

Wilson<sup>4</sup> and others in the U. S. Food and Drug Administration have used immiscible solvent extractions for the separation of glycols and glycol ethers from certain mixtures. Subsequent estimations were made by weighing the extracts. Identifications were made by determining the acetyl value and physical constants such as boiling point, specific gravity, and refractive index. For these determinations considerable material is required, often more than is available from samples of cosmetics.

The usual characterizing derivatives for alcohols are acetate, benzoate *p*-nitrobenzoate, 3,5-dinitrobenzoate, phenylurethan, and  $\alpha$ -naphthyl urethan. For diethyleneglycol-monoethyl ether, these derivatives are apparently all liquids. The scarcity of solid derivatives has therefore made identification difficult by the ordinary procedures.

Mason and Manning<sup>5</sup> have reviewed the literature on derivatives of the monoalkyl ethers of ethyleneglycol and diethyleneglycol. For solid derivatives of definite melting point they proposed the picrates of the  $\beta$ -4-morpholinoethyl ethers, the *p*-nitrophenyl urethans, and other complex compounds.

Previously, Whitmore and Lieber<sup>6</sup> suggested the potassium xanthates as suitable solid derivatives for alcohols, including some monoalkyl ethers of the glycols. Although they proposed the xanthates only as tests for identity, it was on the basis of the xanthate reactions that the quantitative method here described was developed.

<sup>1</sup> Modern Cosmetics by Francis Chilson, 2nd ed. (1938), pp. 125, 152, 250. Drug & Cosmetic Industry, New York.

<sup>2</sup> Trade-mark name—Carbide & Carbon Chemicals Corp., New York.

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<sup>4</sup> Private communication.

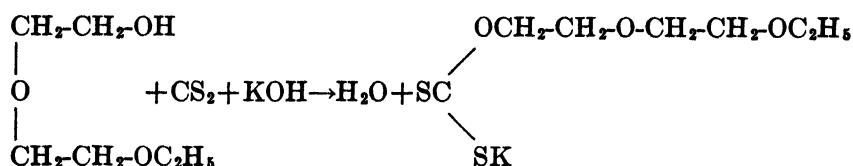
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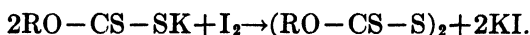
## PRINCIPLES OF METHOD

Carbon bisulfide in the presence of an alkali reacts with many organic compounds containing an hydroxyl functional group to form the so-called xanthates.

In the proposed procedure excess carbon bisulfide and potassium hydroxide are used to cause a quantitative conversion of diethyleneglycol-monoethyl ether to the corresponding xanthate.



The xanthate is extracted by the use of suitable solvents, dissolved in water, and titrated with standard iodine solution. The titration is based on the following reaction, where R stands for the diethyleneglycol-monoethyl ether or other alcohol radical.



Since the xanthate is quite readily isolated and purified, the melting point and iodine equivalent or molecular weight may be determined as suggested by Whitmore and Lieber.<sup>6</sup>

## METHODS

## REAGENTS

- (a) *Potassium hydroxide*.—Reagent grade with minimum of 85% KOH.
- (b) *Acetone-benzene mixture*.—Mix equal volumes of alcohol-free acetone and benzene.
- (c) *Carbon disulfide*.—Reagent grade, alcohol free.
- (d) *Petroleum benzin*.—U.S.P., B.P. 35–80°C.
- (e) *Potassium iodide*.—Reagent grade.
- (f) *Iodine*.—0.05 N. Dilute an accurately measured portion of U.S.P. 0.1 N iodine solution with water to exactly twice its original volume.
- (g) *Starch solution*.—Dissolve 0.5 gram of soluble starch in 100 ml. of boiling water.

## QUANTITATIVE PROCEDURE

Place 10 ml. of an aqueous solution containing 25–250 mg. of diethyleneglycol-monoethyl ether in a separatory funnel. Add 10 grams of solid KOH and cool under a stream of water during solution. Add ca. 5 ml. of the CS<sub>2</sub> and shake for ca. 1 minute. Permit gases to escape by opening the stopcock cautiously. Shake at intervals for 5 minutes and extract with four 15 ml. portions of the acetone-benzene mixture. In making the extractions, draw off the lower alkaline layer into a second separatory funnel, pour the acetone-benzene layer from the top onto a pledget of absorbent cotton (ca.  $\frac{1}{2}$  gram) placed in an ordinary long-stemmed funnel inserted through the top of a third separatory funnel. Wash the orifice of the first separatory funnel with a little solvent mixture and wash out with the next 15 ml. portion of solvent. Drain the solvent into the second separatory funnel, shake thoroughly, and continue as before until the four extractions have been made. Discard the alkaline layer. Wash

the cotton and funnel stem with a little solvent mixture and add ca. 30 ml. of the petroleum benzin to the combined filtered extracts. Extract the organic solvent mixture with a minimum of three 25 ml. portions of water. Drain the water layers into a titration flask and add enough water to make 200 ml. volume. (A drop of phenolphthalein indicator may be added to check the absence of KOH.) Add ca. 2 grams of the KI and 5 ml. of the starch indicator. Titrate with the iodine solution to a blue end point. (Owing to the presence of acetone the end point gradually fades.)

1 ml. of 0.05 *N* iodine = 6.7 mg. of diethyleneglycol-monoethyl ether ( $C_4H_{10}O_2$ ).

#### IDENTIFICATION

Prepare the xanthate from 200–500 mg. of diethyleneglycol-monoethyl ether by the method described under the quantitative procedure. Maintain the (1+1) proportion of KOH in the preparation. Make extractions with two 15 ml. portions of the acetone-benzene mixture. Filter the extracts through cotton into a beaker. Add 50 ml. of the petroleum benzin, stir, and let stand until most of the viscous xanthate has collected on the bottom of the beaker. Pour off the solvents and wash with a little petroleum benzin.

Dissolve the viscous xanthate in ca. 2 ml. of anhydrous amyl alcohol and add 15 ml. of the  $CS_2$ . Cool in a mixture of ice and salt to ca.  $-5^\circ C$ . and add small portions of anhydrous ethyl ether, stirring to induce crystallization. Collect the crystalline xanthate in a sintered glass filter crucible, wash with ethyl ether, and keep covered with a layer of ether to prevent absorption of moisture. Dry the precipitate by aspirating air (previously dried by passage through  $H_2SO_4$ ) through the crucible. Diethyleneglycol-monoethyl potassium xanthate, crystallized in this manner, melts at  $86^\circ$ – $88^\circ C$ .

Transfer an accurately weighed portion of the dried xanthate (ca. 0.3 gram) to a flask, and dissolve in 200 ml. of water. Add the starch indicator, and titrate to a blue end point with the iodine.

Calculate the iodine equivalent as follows:

$$I \text{ (equiv.)} = \frac{\text{ml. of 0.05 } N \text{ Iodine} \times 6.346}{\text{Wt. of xanthate in grams}}$$

The theoretical iodine equivalent of diethyleneglycol-monoethyl potassium xanthate is 511.1.

The molecular weight of the alcohol portion of the xanthate may be calculated as follows:

$$\text{Mol. wt. of alcohol in xanthate} = \left[ \left( \frac{\text{mg. of xanthate} \times 20}{\text{ml. of 0.05 } N \text{ iodine}} \right) - 114.2 \right]$$

The molecular weight of diethyleneglycol-monoethyl ether ( $C_4H_{10}O_2$ ) is 134.1.

#### EXPERIMENTAL

*Preparation of pure diethyleneglycol-monoethyl ether.*—Fractional distillation of commercial specimens of diethyleneglycol-monoethyl ether failed to yield pure distillates. Practical diethyleneglycol-monoethyl ether and "carbitol solvent" showed about 73 per cent of the glycol mono ether by the xanthate titration. The balance consisted chiefly of ethylene glycol. The mixture appeared to have a constant boiling point at  $195^\circ$ – $196^\circ C$ .

Extractions with immiscible solvents were used for the preliminary separation of the glycol mono ether from other glycol impurities.

An ether solution of the commercial glycol mixture was extracted with several portions of aqueous (1+1) potassium hydroxide solution. Ethylene glycol is removed by the aqueous alkali. The resulting ether solution, which retained considerable potassium hydroxide, was neutralized with hydrochloric acid and heated on a steam bath to remove the ethyl ether. Chloroform was then added to the evaporated residue and the solution was decanted from the sludge of salt. The chloroform solution, dried with anhydrous sodium sulfate, was filtered and distilled at atmospheric pressure. The portion boiling at 200°–203° C. was redistilled under forced reflux. The fraction distilling at 201.9°–202.1° C. was collected and used as the reference standard for diethyleneglycol-monoethyl ether. Physical constants were:

Boiling range	201.9–202.1° C.
Specific gravity $\frac{20^{\circ} \text{ C.}}{20^{\circ} \text{ C.}}$	0.9892
Refractive index at 20° C.	1.4266

A second fractionation produced no change in physical properties.

Based on 100 per cent purity for the reference standard, assays by the xanthate titration showed an average recovery of 99.2 per cent.

*Conversion to xanthate and extraction.*—The effect of variations in the quantities of potassium hydroxide and carbon bisulfide was studied to determine suitable conditions for quantitative conversion to the xanthate.

Preliminary experiments showed that a high concentration of alkali is necessary for the rapid formation of the xanthate. A variation of the quantity of 85 per cent potassium hydroxide from 9 to 15 grams for each 10 ml. of water gave no significant difference in recoveries. With 5 grams of alkali, only about 50 per cent recovery was obtained, however.

Sufficient excess of carbon bisulfide was used to cause the separation of the xanthate as a third layer. Small quantities of carbon bisulfide or an excess of diethyleneglycol-monoethyl ether gave lower recoveries.

A time interval is necessary for the completion of the reaction. A more rapid conversion takes place in the presence of a large excess of alkali. With a 5 minute time interval, however, the effects of varying alkali concentrations were equalized. These observations are summarized in Tables 1–5.

A minimum alkalinity was desirable to make the method more specific. With the conditions stated here, glycerin and ethylene glycol did not interfere, but with a greater concentration of alkali they too formed some xanthates even at room temperature.

The potassium 2( $\beta$ -ethoxyethoxy)-ethyl xanthate separates under many conditions as a viscous liquid difficult to crystallize. The dry material, however, gradually crystallizes on long standing or stirring. The liquid is bright yellow with a faint disagreeable odor and is soluble in alcohols,

TABLE 1.—*Effect of KOH concentration on recovery by xanthate titration*

EXP. NO.	DIETHYLENEGLYCOL-MONOMETHYL ETHER CONTAINED IN 10 ML. OF WATER	POTASSIUM HYDROXIDE* ADDED	RECOVERY
	mg.	grams	per cent
1	100	5	47.5
2	100	9	99.3
3	100	10	99.3
4	100	12	99.1
5	100	15	99.1

5 ml. of CS<sub>2</sub> used and a 5 minute time interval.

\* KOH (pellets) assayed 85.3 % of KOH and 4.0 % of K<sub>2</sub>CO<sub>3</sub>.

TABLE 2.—*Effect of time interval on recovery*

EXP. NO.	DIETHYLENEGLYCOL-MONOMETHYL ETHER CONTAINED IN 10 ML. OF WATER	TIME INTERVAL*	RECOVERY
	mg.	minutes	per cent
1	100	1	93.5
2	100	3	99.3
3	100	5	99.3
4	100	10	99.1

5 ml. of CS<sub>2</sub> and 10 grams of KOH used in above experiments.

\* In each case the mixture was shaken for 1 minute after the addition of CS<sub>2</sub> and intermittently during any remaining time.

TABLE 3.—*Effect of varying quantities of CS<sub>2</sub> on recovery*

EXP. NO.	DIETHYLENEGLYCOL-MONOMETHYL ETHER CONTAINED IN 10 ML. OF WATER	CS <sub>2</sub> ADDED	RECOVERY
	mg.	ml.	per cent
1	50	1	98.9
2	100	1	98.1
3	200	1	95.5
4	100	5	99.3
5	200	5	99.4
6	100	10	99.1

10 grams of KOH and a 5 minute time interval used.

acetone, chloroform, and water. It is insoluble in carbon bisulfide, carbon tetrachloride, ethyl ether, and petroleum benzin.

Treatment of the liquid xanthate with a mixture of amyl alcohol, carbon bisulfide, and ether, as directed in the method, gave a voluminous pale yellow crystalline product that melted at 86°–88° C. Recrystallization did not change the melting point. The iodine equivalent and calcu-



TABLE 4.—*Effect of varying volumes of alkaline solution on recovery*

EXP. NO.	DIETHYLENEGLYCOL-MONOETHYL ETHER IN WATER SOLUTION	VOLUME OF WATER* SOLUTION TO WHICH WAS ADDED AN EQUAL WEIGHT OF KOH	RECOVERY
	mg.	ml.	per cent
1	100	10	99.3
2	100	20	99.1
3	100	30	99.4
4	200	10	99.4
5	200	30	99.2
6	198.0	10	99.2
7	228.3	30	99.2

5 ml. of CS<sub>2</sub> and a 5 minute time interval used.

\* Experiments 1-5, inclusive, were made by adding a weight of solid KOH equivalent to the volume of water solution of the glycol ether. The KOH increased the final volume by ca. 50% in each case. Experiments 6 and 7 were made by adding weighed quantities of the glycol ether to the designated volumes of cold (1+1) KOH.

TABLE 5.—*Recoveries by xanthate titration on varying quantities of diethyleneglycol-monoethyl ether*

EXP. NO.	DIETHYLENEGLYCOL-MONOETHYL ETHER CONTAINED IN 10 ML. OF WATER	RECOVERY
	mg.	per cent
1	25	99.2
2	50	99.0
3	100	99.3
4	200	99.4
5	250	99.3
6	300	99.0
7	400	98.4
8	500	97.3
9	None—blank	0.0

5 ml. of CS<sub>2</sub>, 10 grams of KOH, and a 5 minute time interval used.  
(Proposed limits for the method are 25-250 mg. of the glycol ether.)

lated molecular weight of the crystalline material corresponded to the theoretical values.

Whitmore and Lieber reported obtaining a liquid xanthate from "carbitol," which upon long drying gave a pasty solid melting at 127° C. The reported physical properties of their diethyleneglycol-monoethyl ether indicate that they were using an impure product probably containing ethylene glycol. In their method of preparation of the xanthates, such ethylene glycol would cause interference.

The effectiveness of various solvents and solvent mixtures for removal of the xanthate from the alkali was studied. A mixture of acetone and benzene was found to satisfy the requirements of solubility and selectivity. Both reagents are easily obtainable with sufficient purity to give negligible blanks.

The absence of even traces of potassium hydroxide in the extract is necessary for the proper functioning of the method. The filtration through absorbent cotton, described in the method, seems effective since no carry-over of potassium hydroxide was observed in more than 100 extractions.

Petroleum benzine is added to the filtered extracts to prevent emulsification and to obtain a more rapid extraction with water.

The water extract of the xanthate before titration with iodine contains most of the acetone used in the extractions. Preliminary tests showed an appreciable blank due to the presence of the acetone. The addition of potassium iodide reduced this blank from 0.3 ml. to less than 0.05 ml. of

TABLE 6.—*Effect of distillation on recovery of diethyleneglycol-monoethyl ether*

DIETHYLENGLYCOL-MONOEETHYL ETHER CONTAINED IN 100 ML. OF WATER	DISTILLED AT—	DISTILLATE	GLYCOL ETHER RECOVERED—		
			IN DISTILLATE		IN RESIDUE
mg.			mg.	per cent	per cent
426.9	Atmospheric pressure	1st 25 ml.	11.4	2.67	
		2nd 25 ml.	15.8	3.70	
		3rd 25 ml.	24.2	5.67	
		Total 75 ml.	51.4	12.04	87.1
426.9	45 mm. pressure	75 ml.	3.7	0.86	98.1

0.05 *N* iodine for 30 ml. of acetone in 200 ml. of water. Experiments with weighed quantities of the purified xanthate showed no interference due to the presence of acetone and potassium iodide. The end point is stable for 2 or 3 minutes, but gradually fades on longer standing.

*Separation from mixtures.*—Since many primary and secondary alcohols form xanthates, preliminary separations are often necessary in mixtures. In cosmetics the more common interfering substances include ethyl and isopropyl alcohols, glycols, and ethanolamines. These are water-soluble materials that, like diethyleneglycol-monoethyl ether, are not ordinarily extracted by immiscible solvents from dilute aqueous solutions.

Low-boiling alcohols (such as ethyl and isopropyl) may be removed, preferably by a vacuum distillation (see Table 6). They are incompletely converted to the corresponding xanthates under the specific conditions of the method, and their xanthates are only slightly soluble in the acetone-benzene mixture. Suitable conditions could probably be developed for their estimation by a xanthate titration, however.

Glycerin, ethylene glycol, and sorbitol showed no interference in the method. Commercial specimens of propylene glycol, diethylene glycol, and the ethanolamines gave small and varying titrations. For  $\frac{1}{2}$  gram quantities of the latter two glycols 0.5–2.0 ml. of 0.05 *N* iodine was required.

The mono-, di-, and triethanolamines appeared to be partially extracted as the bases and gave indefinite end points with iodine. At elevated temperatures and with concentrations of alkali greater than specified in the method, the ethylene, propylene, and diethylene glycols formed crystalline xanthates insoluble in acetone.

An extraction, similar to that used for purification of the glycol ether, was used to eliminate the interfering glycols and ethanolamines. A solvent composed of equal parts of ethyl ether and petroleum benzin was used instead of ether alone. Small quantities of the glycol ether were completely

TABLE 7.—*Recovery of diethyleneglycol-monoethyl ether from mixtures*

EXP. NO.	MIXTURE CONTAINED—	DIETHYLENEGLYCOL- MONOETHYL ETHER	
		ADDED	RECOVERED
1	Ethyleneglycol 100 mg. in 10 ml. of water	mg 200	per cent 99.1
2	Ethyleneglycol 100 mg. Glycerin 100 mg. Sorbitol 100 mg. } in 10 ml. of water	200	98.9
3	Ethyleneglycol 100 mg. Glycerin 100 mg. Triethanolamine 100 mg. Diethyleneglycol 100 mg. Propyleneglycol 100 mg. } in 10 ml. of water	200	97.0
4	Vanishing cream 5 grams	112.7	95.0
	per cent		
	Stearic acid. . . . .	24.0	
	KOH. . . . .	0.5	
	Triethanolamine. . . . .	0.5	
	Glycerin. . . . .	2.0	
	Ethyleneglycol. . . . .	2.0	
	Propyleneglycol. . . . .	2.0	
	Sorbitol. . . . .	2.0	
	Water. . . . .	67.0	
5	Vanishing cream 5 grams	0.0	0.0
6	Lotion, 50 ml.	500	96.8
	per cent		
	Alcohol. . . . .	50.0	
	Quinine sulfate. . . . .	0.5	
	Salicylic acid. . . . .	0.5	
	Water. . . . .	49.0	

extracted from a (1+1) aqueous potassium hydroxide solution by this solvent mixture; and ethanolamines, propylene glycol, ethylene glycol, diethylene glycols, and glycerin were left behind. The proportion of solvent mixture to glycol ether must, however, be about 100 to 1 to prevent the formation of a third layer. For this reason the solvent is unsuitable for purification or extraction of large quantities of the glycol ether.

Other glycol monoethers would be extracted and converted to the corresponding xanthates. Their different xanthate melting points and iodine equivalents<sup>6</sup> would distinguish them, however, from carbitol.

In recovery Experiments 1 and 2 (Table 7), determinations were made in the presence of the ethyleneglycol, glycerin, and sorbitol. In the remaining experiments preliminary separations were made.

In Experiment 3, the glycol ether was extracted with a (1+1) petroleum benzin-ethyl ether solvent from the aqueous solution after addition of an equal weight of potassium hydroxide. The glycol ether was then re-extracted from the solvent with water and determined by the method applied to the water solution.

In Experiments 4 and 5, the weighed samples of vanishing cream were heated in the presence of benzene and dilute acetic acid to dissolve the stearic acid and break the emulsion. The acid aqueous extract was made alkaline with an equal weight of potassium hydroxide and extracted with (1+1) petroleum benzin-ethyl ether solvent to isolate the glycol ether. The final re-extraction with water and determination were made as in Experiment 3.

In Experiment 6, the sample was diluted with an equal volume of water and distilled under vacuum to about  $\frac{1}{2}$  the original volume to remove the alcohol. Salicylic acid and quinine were removed by ether extractions from the acidified (acetic) and alkalized (KOH) residue. The aqueous residual solution containing the glycol ether was made to volume with water, and an aliquot part taken for the final determination.

#### SUMMARY

Procedures suitable for the determination and identification of diethyleneglycol-monoethyl ether in cosmetics are described. The methods are based on the conversion of the glycol ether to its potassium xanthate, extraction of the xanthate, and titration with iodine.

The effects of variations in the reagents and the influence of other alcohols on recoveries were studied.

Typical recovery data on mixtures of known composition are included.

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## LABORATORY STUDIES OF OYSTERS PREPARATORY TO THE FORMULATION OF STANDARDS

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In the preparation of a field program in connection with the project of establishing a definition and standard of identity for shucked oysters, it was necessary to do the experimental work reported in this paper in order to supplement the available information on oysters, which for various reasons was inadequate. The limited time available made it necessary to restrict the work to several specific subjects on which facts were lacking. The findings and their significance are presented, and the data on which they are based are given in the experimental part of the paper.

### VARIATION IN COMPOSITION OF OYSTERS WITHIN A LOT

Analyses of individual shell oysters and others taken in groups of five indicate that there is a wide variation in the solids and the salt content as well as in the weight of individual oysters. Statistical treatment of such analyses of two lots of oysters of different types indicates that the percentage of solids in one quart samples may differ from the mean percentage solids of the lot by more than 0.3 per cent one time out of three, and by more than 0.6 per cent one time out of twenty. It was also found that no correlation exists between the size of the oyster and the salt or solids content. Although these oysters were not known to have come from the same bed, from their appearance there was no reason to doubt that those in a single lot were from the same area. The variation noted is certainly representative of conditions frequently encountered in commercial practice, where there is no segregation of lots from widely scattered beds. On the other hand, the consistency in the results previously obtained with one quart samples taken from lots of shucked-out oysters suggests that in such a composite an equilibration process occurs and renders the composition of individual oysters more uniform. Such an equilibration would be expected on theoretical grounds.

The variations in composition found in this work make the sampling problem so complex as to make impracticable on a commercial scale the utilization of changes in the solids content as a measure of the absorption of water during the washing process.

### RELATION OF SHUCKING PROCEDURE TO EXTENT OF BLEEDING AND CLEANLINESS OF OYSTERS

To justify the commercial practice of shucking oysters into water, it has been claimed that the water decreases the so-called bleeding (exudation of oyster liquid after shucking) and makes it easier to clean the oysters. This bleeding is so copious that it might cause a serious loss of

oyster solids. In order to determine the effect on loss of solids during shucking and on the ease of cleaning, three procedures were compared: Shucking oysters (1) into dry pots; (2) into pots containing water; and (3) into perforated pots that permit the liquor to run off. The results show that only a small quantity of solids is lost by any of these procedures and that the loss is as great by shucking into water as by the other methods. However, the yield of oyster meats is greater from shucking into water owing to the absorption of water while the oysters are in the shucking pot so that the result is an apparent decrease in the loss from bleeding.

Oysters shucked by the above methods were blown for three minutes in fresh water and compared as to cleanliness. While the objective method of comparison used does not permit close distinctions, it showed definitely that the oysters shucked into water were cleaner than those shucked into dry or perforated pots. The dry pots also seemed to yield cleaner oysters than did the perforated pots. Of the oysters having rather dirty shells, the ones shucked into dry or perforated pots did not appear to be sufficiently cleaned by the three minute blow. On the other hand, oysters shucked in any of the three ways from clean shell stock yielded a cleaner product than that obtained by shucking dirty shell stock into water. These findings are in accord with the unpublished results of similar studies made in the field a number of years ago and indicate that if the shucking of dirty shell stock can be justified at all, the use of a limited quantity of water in the shucking pot may serve to minimize the extent of washing necessary.

#### MAXIMUM WATER-ABSORPTIVE CAPACITY OF OYSTERS

It has been suggested that under controlled conditions oysters might exhibit a characteristic maximum water-absorptive capacity that would permit conclusions as to how much water a given lot of oysters had already absorbed from the additional quantity they would take up under the controlled conditions.

A study of such conditions showed that oysters will not absorb their maximum quantity of water on blowing. It is difficult to determine their maximum absorption in either still or running water because when holding excessive quantities they cannot be handled without loss of water, which loss is apparently not regained on further soaking. No relation was observed between the percentage of water absorbed and the solids or salt content or the size of the oysters. Any differences in absorptive capacity due to temperature differences (8°–21° C.) were exceeded by the variations in behavior of individual samples (approximately one quart) from the same lot. There is evidently a marked variation among different lots of oysters in the rate as well as in the degree of absorption of water. It was concluded that no objective method for estimating the quantity of water that oysters have already absorbed can be developed on the basis of the additional quantity that they will absorb under uniform conditions.

### MEASUREMENT AND DEVELOPMENT OF FREE LIQUID

A study was made of various methods of determining free liquid. Since any method for this purpose is necessarily empirical, it is difficult to establish accuracy. The use of the perforated tray type of skimmer yielded as precise results as can be expected. However, it was shown that the free liquid should be determined on a weight basis in preference to the volume basis now used. The free liquid is best estimated from the difference in weight of the oysters before and after drainage under specified conditions. Since sampling is a major source of error in such determinations, the entire sample (not less than one gallon) should be used, either by applying the method to successive quarts and taking the average or possibly by using a skimmer of the proper size to accommodate the entire sample.

Some of the washed oysters were held for a time under various conditions to determine the relative quantities of free liquid they would develop. The results indicate that oysters that have absorbed excessive quantities of water may develop significant quantities of free liquid on storage. These results are in accord with data reported in U. S. Department of Agriculture Technical Bulletin No. 64. Recent unpublished studies by the Baltimore Station of the U. S. Food and Drug Administration indicate that oysters that have been properly washed and drained do not develop an excessive amount of free liquid on one week's storage in ice.

Because it has been claimed that oysters from the Apalachicola section of Florida tend to bleed excessively and develop substantial quantities of free liquid during storage and shipment, two lots of these oysters were studied. They were treated in exactly the same way as was a lot of Chesapeake Bay oysters of practically the same salt and solids content. The results are not conclusive, but they show the tendency claimed.

### CHANGE IN COUNT AS A MEASURE OF OVER-RUN

The study of the practicability of using the decrease in count per gallon as a measure of the increase in volume or weight (over-run) was necessarily restricted in the laboratory experiments. The limited data obtained on straight shuck (ungraded) oysters indicate that the variation of the count in successive quarts of such oysters is so great as to lead to false conclusions if the count were to be used as a measure of over-run. This variation would undoubtedly be much less on gallon samples within a given size designation, but further studies of the variation were not made, since the more satisfactory direct measurement of over-run can be readily made in the packing plant.

### EXPERIMENTAL

With the exception of the two lots from Apalachicola, Florida, the shell oysters used were purchased from a reliable Washington dealer. In nearly

all cases they were obtained within a few hours after delivery to the dealer, who said that they had been out of the water not more than two or three days.

*Variation in Composition of Oysters within a Lot*

*Sample No. Inv. 71203-D—Milford Haven, Va.*—Thirty individual oysters were shucked, allowed to drain 5–10 seconds, weighed, and passed through a Wiley mill (20-mesh screen) in tandem, the ground material being collected separately for each oyster. Total solids and salt were determined. Since variable amounts of each oyster were retained in the mill there was a leveling effect that reduced the individual variations and made it impossible to study the correlation between solids and salt content and weight of the oyster in this series. The data are summarized in Table 1.

TABLE 1

	WEIGHT	SOLIDS	SALT
	grams	per cent	per cent
Maximum	28.5	18.06	1.16
Minimum	11.2	8.95	0.83
Mean	19.45	14.71	0.94
Standard deviation		2.206	

This lot of oysters averaged 49 to the quart. On this basis for normal distribution the percentage of solids determined on a one quart sample would be expected to differ from the mean solids content of the lot by more than  $\pm 0.32$  per cent one time out of three and by more than  $\pm 0.64$  per cent one time out of twenty. These oysters were picked from a barrel of shell stock as delivered, without mixing of the lot. In order to confirm the extent of the variation and to determine the correlation between solids and salt content and the weight of the oyster, another lot was sampled and analyzed as described below.

*Sample No. Inv. 71204-D—Chincoteague Islands.*—Two bushels of shell stock were completely randomized by distribution into lots of 100 oysters each. The oysters from one lot were shucked, drained 5–10 seconds, and then weighed and analyzed in groups of five. Each group was passed through a Wiley mill (20-mesh screen) twice, and the mill was cleaned after each group had been prepared for analysis. The twenty samples thus prepared were analyzed for solids and salt content. The data are shown in Table 2.

This lot of oysters averaged 45 to the quart. On this basis for normal distribution the percentage solids determined on a one quart sample would be expected to differ from the mean solids content of the lot by more than  $\pm 0.34$  per cent one time out of three and by more than  $\pm 0.68$  per cent one time out of twenty.



TABLE 2

GROUPS OF FIVE

GROUP NO.	WEIGHT	SOLIDS	SALT
	<i>grams</i>	<i>per cent</i>	<i>per cent</i>
1	119.0	20.25	1.36
2	112.0	19.56	1.37
3	101.0	17.82	1.48
4	100.7	17.92	1.50
5	97.7	19.16	1.40
6	112.7	17.29	1.52
7	100.2	17.98	1.42
8	101.2	19.12	1.38
9	105.5	19.88	1.35
10	100.5	18.66	1.40
11	100.7	18.56	1.43
12	113.0	18.79	1.48
13	115.7	19.89	1.41
14	104.0	17.65	1.54
15	98.0	20.66	1.30
16	106.0	19.20	1.38
17	90.5	20.40	1.34
18	109.2	19.21	1.40
19	106.0	20.39	1.27
20	108.5	19.58	1.38
Maximum	119.0	20.66	1.54
Minimum	90.5	17.29	1.27
Mean	105.0	19.10	1.41
Standard deviation		1.013	

The correlation coefficients between the solids content and the weight of the oyster ( $r=0.036$ ) and between the salt content and the weight of the oyster ( $r=0.173$ ) are both insignificant.

*Relation of Shucking Procedure to Extent of Bleeding and Cleanliness*

*Sample No. Inv. 71203-D—Milford Haven, Va.*—In the initial experiment in this series a quart of oysters was shucked into a dry quart measure, counted, and weighed; the oysters were drained two minutes on a 14-inch 8-mesh sieve, the drained weight was determined, and the drained liquid was returned to the oysters after a sample had been withdrawn for analysis. The draining and weighing were repeated at intervals during 24 hours, when the drained oysters and the liquid were separately analyzed for solids and salt content. At the same time, an equal number of oysters was shucked into one quart of water and treated in like manner. The sampling error, the unavoidable losses of liquid, etc., involved in the repeated handling, prevented any reasonable comparison of the progressive changes occurring, but the over-all differences have some significance.

In Table 3 under Inv. 71203-D, the comparison of the losses in weight, solids, salt, and salt-free solids of the oysters is shown. The "original" condition refers to the oyster immediately after shucking and allowing adhering shell liquid to drip off for about 5 seconds. There is also shown the comparable loss by bleeding when ten oysters were shucked upon an 8-mesh sieve and allowed to drain freely for 18 hours. The composition of the shell liquor and of the liquid exuded from the oysters during the first half hour, the next hour, and the following 16½ hours is shown in Table

TABLE 3

SAMPLE	WEIGHT PER 100 OYSTERS LBS.	SOLIDS PER CENT	SALT PER CENT	WT. PER 100 OYSTERS OF			DECREASE IN ORIGINAL			
				SOLIDS LBS.	SALT LBS.	SALT- FREE SOLIDS LBS.	WEIGHT PER CENT	SOLIDS PER CENT	SALT PER CENT	SALT- FREE SOLIDS PER CENT
Inv. 71203-D										
Original	4.29	14.71	0.94	0.631	0.040	0.591	—	—	—	—
In dry pot	2.97	19.48	0.72	0.580	0.022	0.558	31	8	45	6
In water	3.96	14.67	0.37	0.580	0.016	0.564	8	8	60	5
On sieve*	3.00	22.41	0.66	0.672	0.020	0.652	39	7	57	3
Inv. 71204-D										
Original	4.63	19.10	1.41	0.884	0.065	0.819	—	—	—	—
In dry pot	3.62	22.70	1.13	0.822	0.041	0.781	22	7	37	5
In water	3.84	19.93	0.76	0.766	0.029	0.737	17	14	56	10
In perforated pot	3.58	23.24	1.08	0.832	0.039	0.793	23	6	40	4

\* Only 10 oysters were used in this experiment; they weighed 4.90 lbs. per 100 (4.29 lbs. were used in the preceding calculations as the original weight).

4. It is interesting to note that the great bulk of the exuded liquid drains during the first half hour.

*Sample No. Inv. 71204-D—Chincoteague Island.*—To more nearly simulate plant practice, another experiment was made in which one quart samples were shucked into a dry pot, into a pot containing one quart of water, and into a perforated pot that permitted liquid to run off; in each case the oysters were held just one hour from the beginning of shucking before they were drained on an 8-mesh sieve, weighed, and analyzed. The data are also shown in Table 3 under Inv. 71204-D.

Because of the sampling uncertainty involved in comparisons of this type, too much reliance should not be placed on the absolute values obtained. In fact, in a later series of similar experiments with Apalachicola oysters, the sampling error was such as to yield absurd results. However, the above experiments are adequate to show (1) that the apparent decrease in bleeding on shucking into water is due to replacement of some of the exuded oyster liquid by water; (2) that the actual loss of oyster solids (both salt-free solids and salt) on shucking into water is not less than the loss on shucking into dry or perforated pots; and (3) that the

loss of solids is small in any case in spite of the large losses of oyster liquid.

The study of the effect of the shucking procedure on the cleanliness of oysters is handicapped by the lack of any objective measure of cleanliness. However, an attempt was made to obtain some comparative results on oysters shucked into water, into dry pots, and into perforated pots. Approximately one quart of oysters was used in each case. The wet-shucked

TABLE 4

INV. 71203-D	QUANTITY AS PERCENTAGE OF ORIGINAL WEIGHT	SOLIDS	SALT
		<i>per cent</i>	<i>per cent</i>
Shell liquor	—	2.77	1.53
Liquid exuded in first $\frac{1}{2}$ hour	22	3.25	1.55
Liquid exuded in next hour	3	3.45	1.50
Liquid exuded in next $16\frac{1}{2}$ hours	3	4.80	1.65

oysters were shucked into one quart of water. In all three cases the oysters were held one hour from the beginning of shucking, drained on an 8-mesh sieve, blown 3 minutes in a gallon of water, and again drained. The procedure used to determine the comparative cleanliness was as follows: From each of the three samples one oyster was picked at random and placed on a glass plate; the three oysters were ranked by inspection with a reading glass as 1, 2, 3, No. 1 being the cleanest and No. 3 the dirtiest; this procedure was repeated until all the oysters had been so ranked (alternate sets were ranked by different analysts); and the total number in each rank for each sample was noted. The results are shown in Table 5.

TABLE 5

SHUCKED—	INV. 71203-D			INV. 71204-D		
	1	2	3	1	2	3
In dry pot	9	12	26	8	16	1
In water	30	14	3	11	7	5
In perforated pot	8	19	18	5	1	18

The shells of the Milford Haven oysters (Inv. 71203-D) were rather dirty, while those of the Chincoteague oysters (Inv. 71204-D) were relatively clean. The differences in cleanliness between the subdivisions of Chincoteague oysters shucked in the different ways were comparatively small. In fact, in four of the sets of three the three oysters were so similar in appearance that they could not be ranked 1, 2, 3, as to cleanliness. On the whole, the dirtiest of the oysters from the Chincoteague lot were cleaner than the cleanest ones of the Milford Haven lot. The Milford Haven samples that had been shucked into a dry pot or into a perforated

pot were not regarded as adequately cleaned by the 3-minute blow. It is evident from the data that oysters shucked into water are much cleaner than those shucked into a dry pot or a perforated pot and that those shucked into a dry pot are somewhat cleaner than the ones shucked into a perforated pot.

The excessive proportion of water used in these experiments was intended to bring out more distinctly differences that might have been otherwise obscured because of the inadequacy of the methods of comparison available.

#### *Maximum Water-Absorptive Capacity of Oysters*

In the initial experiments an attempt was made to determine the maximum water-absorptive capacity of individual oysters. For this purpose there was used a large constant temperature bath equipped with a stirrer and six small wire baskets to support the oysters in the bath. Each oyster

TABLE 6

OYSTER NO.	INITIAL WEIGHT	PERCENTAGE INCREASE OVER INITIAL WEIGHT AFTER					
		½ HOUR	1½ HOURS	5 HOURS	7 HOURS	18 HOURS	24 HOURS
	<i>grams</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	21.3	11.9	—	—	—	50.2	—
2	21.4	18.2	—	—	—	61.2	—
3	19.3	8.3	—	—	—	58.0	—
4	21.2	9.3	—	—	—	54.7	—
5	15.6	31.8	—	—	—	95.5	—
6	17.5	12.6	—	—	—	55.4	—
7	22.3	15.3	25.6	34.4	46.8	—	36.8
8	19.1	18.8	28.2	44.0	46.5	—	49.2
9	25.6	-0.4	9.4	16.0	21.2	—	28.9
10	22.5	14.2	19.1	35.6	35.6	—	29.8
11	16.2	20.4	30.9	48.0	48.0	—	47.0
12	23.4	4.7	22.6	26.1	28.2	—	4.7

was shucked carefully to avoid cutting any part except the abductor muscle, drained for 10 seconds, weighed, and placed in one of the baskets submerged in the bath. From time to time the oyster was removed from the bath, drained for 10 seconds, weighed, and replaced in the bath. The results in Table 6, obtained at 15° C. with oysters from the same lot (Inv. 71204-D—Chincoteague Island, Va.), show the extreme variation in the rate and degree of water absorption observed even under closely controlled conditions. There seems to be no relation between the size of the oyster and its water absorption characteristics.

Subsequent studies of water-absorptive capacity were limited to samples of approximately one quart. They were placed in a one-gallon container provided with a perforated false bottom on which they rested

while fresh running water maintained at constant temperature flowed up through them.

In other experiments a small blower designed for blowing the oysters in a gallon of water was used; the temperature of the water was controlled within a 2° C. range and the degree of agitation was regulated by use of a water manometer on the air line.

Combinations of blowing and soaking were also studied in the attempt to establish conditions under which oysters might absorb any reproducible characteristic quantity of water. The apparent maxima observed under various conditions are given in Table 10 with the concurrent experiments on free liquid development. Only a few typical examples of the behavior observed under various conditions are presented in the graphs, Figures 1, 2, 3, and 4, which represent experiments correspondingly identified in Table 10. In all cases the initial weight was that of the drained oysters obtained by shucking one quart into their own liquor, allowing to stand one hour from the beginning of shucking, and then draining two minutes on a large 8-mesh sieve.

In Figure 1, the solid line represents the weight increase obtained when the oysters were blown in one gallon of water to constant weight; the cross marks indicate when the water was changed for fresh water. After the oysters had reached constant weight, they were soaked in running water, where they absorbed at least again as much water as they absorbed during blowing. The dotted line does not show the course of this absorption on soaking, since other experiments (Fig. 2) indicate that after such soaking the oysters cannot be handled without loss of water and hence the true maximum absorption undoubtedly exceeds the percentage here indicated after 16 hours' soaking.

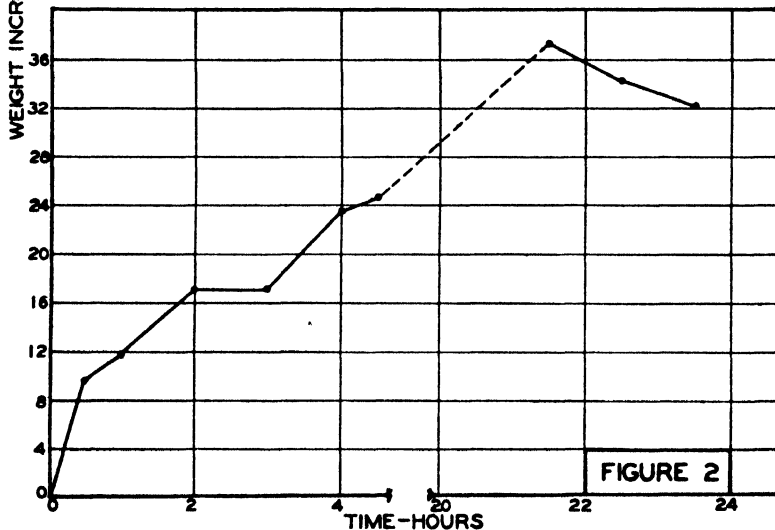
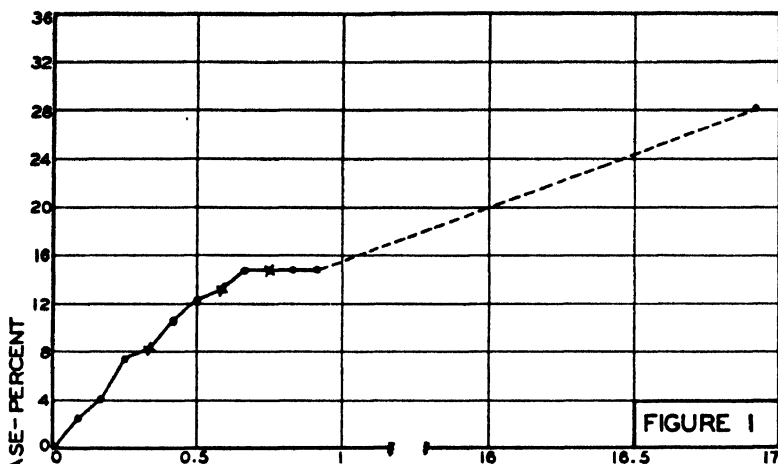
Figure 2 shows the course of the absorption of water when oysters were soaked in running water for long periods. Here again the dotted line represents a long period during which weighings were not made, and it does not represent the course of the absorption. As indicated previously, the maximum shown is probably not the true maximum percentage of water absorbed during this period.

Figure 3 represents the course of the absorption of water when the oysters were blown in a gallon of water to constant weight and the water was changed after each blow. Although constant weight was obtainable under such conditions, the percentage increase was not reproducible even with oysters from the same lot.

The experiment represented in Figure 1 indicates that on blowing oysters do not absorb their maximum quantity of water. Figure 4 indicates that oysters that have absorbed a maximum quantity of water on soaking lose much of this water on blowing. These oysters had been soaked in running water at 3° C. for 45 hours before the blowing experiment represented in Figure 4 was made.

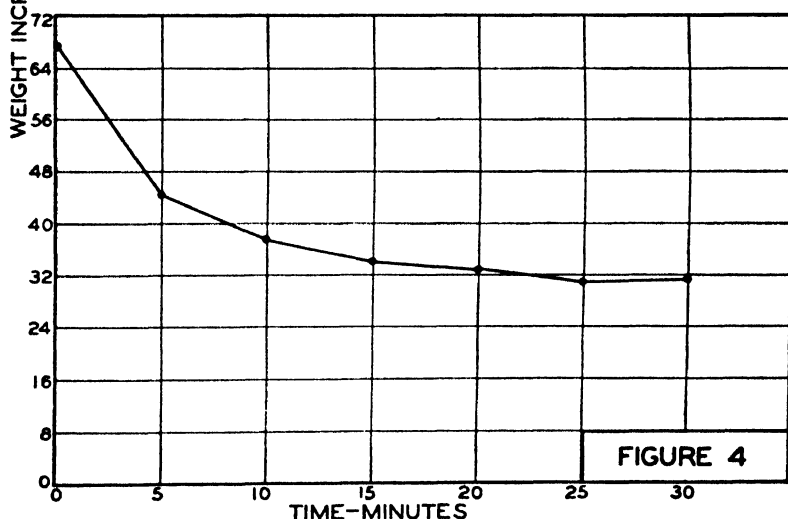
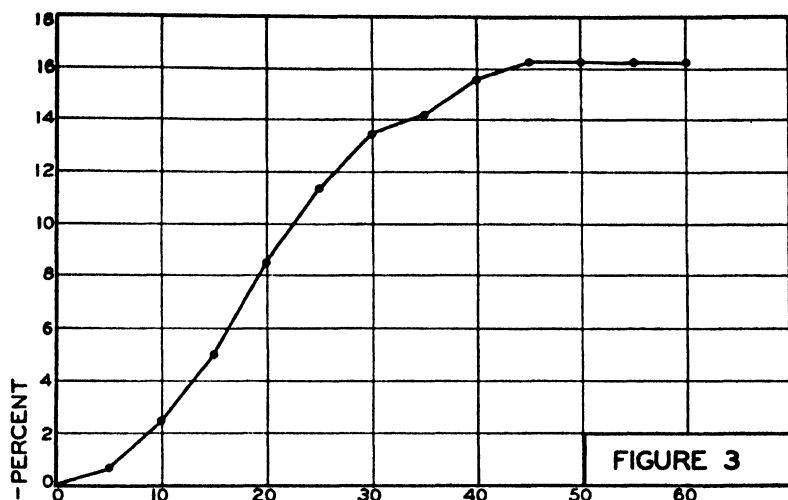
*Measurement and Development of Free Liquid*

An attempt was made to determine the "accuracy" and precision of the present tentative method for the determination of free liquid, *Methods of Analysis, A.O.A.C.*, 1940, XXIX, 2(f), in comparison with a method using an 8-mesh sieve in place of the usual perforated tray. In fact, such



a determination can have no real "accuracy" since the quantity of free liquid in oysters can be defined only in terms of the method used to determine it. The term "accuracy" here refers to the degree to which a definite quantity of liquid added to oysters is indicated by a given method as distinguished from the precision (reproducibility) of that method. It was difficult to determine this accuracy, but some of the accumulated data

indicate the comparative accuracy of the methods in which the sieve and the skimmer, respectively, were used. The oysters (Inv. 71205-D, Milford Haven, Va.) were shucked into an equal volume of water and kept in a



cold room (40° F.) overnight, the object being to obtain oysters as nearly as possible in equilibrium with the surrounding free liquid. They were drained "dry" on the 8-mesh sieve. To approximately one quart of the dry oysters there was added a weighed quantity of the drained liquid. The oysters were fluffed four times by pouring them back and forth from one

beaker to another. (The beakers were first wet with drained liquid and then thoroughly drained.) Their volume was measured in a standard quart measure by means of the dodgometer (depth gauge). They were poured out on the dry sieve or skimmer in a single layer with a minimum of handling (several seconds) and allowed to drain exactly two minutes. They were flipped from the sieve or scraped off of the skimmer into the measure and their volume and weight were again determined. The loss in volume or weight was calculated in terms of the percentage of free liquid. The results (Table 7) indicate that on the weight basis the sieve method yields somewhat higher average results than the "true" amount of free liquid present, though somewhat greater precision is noted for it than for the skimmer method. However, the volume determination is shown to be much less satisfactory than the weight determination by either method.

TABLE 7

SIEVE METHOD FREE LIQUID			SKIMMER METHOD FREE LIQUID		
PRESENT (ADDED)	FOUND		PRESENT (ADDED)	FOUND	
	BY VOLUME	BY WEIGHT		BY VOLUME	BY WEIGHT
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9.1	5.7	9.1	9.1	4.6	7.0
9.1	6.7	9.6	9.1	5.7	8.6
9.1	9.1	10.3	9.0	7.9	10.0
9.1	7.0	10.7	9.1	8.0	9.1
9.1	6.3	9.8	9.1	8.0	9.4
Average					
9.1	7.0 ± 0.88	9.9 ± 0.48	9.1	6.8 ± 1.35	8.8 ± 0.82

Further evidence that the volume measurement by the dodgometer is itself subject to considerable error as applied to quart samples was obtained. Thus one sample measured successively, with fluffing four times between the measurements, yielded the following results: 0.92, 0.91, 0.91, 0.91, and 0.90 quart. The results with another sample were 0.85, 0.83, 0.85, 0.84, and 0.84 quart. Since the free liquid determination by volume is based on the difference between two such volume measurements, it is to be expected that the precision of the determination would be unsatisfactory for this reason alone, aside from the factors associated with its empirical character.

There apparently has been a practice of using only a portion of a composite mixture of oysters for the determination of free liquid. The following experiment shows that such a procedure is not sound. To approximately a gallon of "dry" oysters of the type used in the preceding experiment, a weighed quantity of the drained liquid was added. Immediately after the oysters were thoroughly mixed by fluffing, approximately a



one-quart sample was rapidly poured into a measure and weighed, and the free liquid was determined by the skimmer method as described previously. This procedure was repeated with the remainder of the gallon sample until free liquid had been so determined on the entire sample. The results (Table 8) show that though the average free liquid found was reasonably close to the quantity added, the value obtained on any particular quart varied considerably from that average.

TABLE 8

## SKIMMER METHOD

ALiquot NO.	FREE LIQUID FIRST TRIAL	REPEAT AFTER RESTORING LIQUID LOST IN FIRST TRIAL
	<i>per cent</i>	<i>per cent</i>
1	7.8	7.9
2	5.3	6.6
3	4.6	5.6
4	4.6	5.2
Average	5.9	6.3
Free Liquid Added	6.5 per cent	

Further studies indicate that a draining time of two minutes is no more desirable than the one-minute period. Prior experience had also shown the advisability in the case of oysters stored in ice of bringing them to a temperature of 10°–12° C. before making free liquid determinations. Some question arose as to the importance, with respect to the precision of the determination, of the manner of removing the oysters from the skimmer, and it was also suggested that fluffing might be omitted. To study these factors a pack of oysters was put up in gallon cans in a commercial plant. Some of the oysters were packed commercially dry and others were drained dry and then packed in cans containing a known weight of water (added free liquid). The packed oysters were stored in ice for one week and the free liquid was then determined by three variations in procedure: (1) Fluffing the oysters before draining and scraping the oysters from the skimmer, (2) pouring the oysters on the skimmer without fluffing, and (3) fluffing the oysters before draining and removing them from the skimmer by tilting it so as to cause them to slide off. The results (Table 9) do not show any very marked differences in the precision of the various procedures. Those involving fluffing yield results approaching more closely the added amount of free liquid. Since sliding the oysters off the skimmer by tilting is a method lending itself to less variation in technic than scraping the oysters off, the former procedure is considered preferable for general use.

The decrease in the free liquid on storage of oysters in ice has been often observed in the past in regulatory operations. The small percentage of

TABLE 9

PROCEDURE 1 OYSTERS FLUFFED; REMOVED FROM SKIMMER BY SCRAPING		PROCEDURE 2 OYSTERS NOT FLUFFED; REMOVED FROM SKIMMER BY SCRAPING		PROCEDURE 3 OYSTERS FLUFFED; REMOVED FROM TILTED SKIMMER BY SLIDING	
FREE LIQUID		FREE LIQUID		FREE LIQUID	
FOUND	ADDED WHEN PACKED	FOUND	ADDED WHEN PACKED	FOUND	ADDED WHEN PACKED
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
10.1	12.0	9.2	11.9	10.2	11.9
10.9	11.9	8.3	11.9	9.6	12.0
10.1	11.9	8.9	12.0	10.0	11.9
9.4	12.0	8.7	11.9	9.3	12.0
2.8	0.0			2.9	0.0
3.1	0.0			2.0	0.0
3.5	0.0			2.8	0.0
				2.9	0.0
				2.2	0.0

free liquid noted in the dry packed oysters after storage in ice represents the amount of the viscous surface liquid that adheres to all surfaces that such oysters contact.

The details of the recommended procedure for the determination of free liquid in oysters taken from storage in ice follow:

#### APPARATUS

*Scale*.—Accurate to  $\frac{1}{4}$  of 1% of the gross weight of the unit of packed oysters to be tested.

*Skimmer*.—A rigid flat-bottomed circular tray ca. 14" in diameter. The bottom is smooth and is perforated with holes  $\frac{1}{4}$ " in diameter, the centers of the holes being  $1\frac{1}{4}$ " apart. The side of the tray is not less than 2" high and has an opening 4–5" wide through which oysters may be slid by tilting the skimmer. The skimmer is so supported as to permit free drainage from the perforations.

*Beaker*.—A 1500 ml. beaker having a diameter about  $\frac{2}{3}$  of its height; it is calibrated with a mark at the quart level.

*Funnel*.—A metal funnel with a smooth inner surface, 8–10" in diameter at the top, with a stem 3" in diameter and 3" long and having a height including the stem of 7–8".

*Fluffing vessels*.—Two vessels, each of such size that the sample of oysters to be tested will occupy ca.  $\frac{1}{3}$  of the capacity.

*Weighing vessel*.—A vessel of sufficient capacity to hold the entire sample.

#### DETERMINATION

Allow the packed oysters to warm to 10°–12° C., take a sample consisting of enough units to total not less than one gallon and determine their gross weight. Pour the oysters from all the units into one of the dry fluffing vessels. Fluff the entire sample by pouring the oysters portion-wise through the funnel into the other dry fluffing vessel and then likewise back into the first fluffing vessel containing adhering

oyster liquid. Immediately pour the oysters from the fluffing vessel into the dry 1500 ml. beaker up to the mark, and then from the beaker upon the dry level skimmer in a single layer; if it is necessary to further spread the oysters in order to get them in a single layer, spread them immediately with minimum handling (3-5 seconds) and with no downward pressure. Drain the oysters 1 minute without further handling and transfer into the tared weighing vessel by tilting the skimmer quickly, avoiding any dripping of liquid from the skimmer into the weighing vessel. Immediately repeat the fluffing, measuring, draining, and transferring of successive quart portions of the oysters remaining in the fluffing vessel until the final portion of one quart or less has been so drained. Do not dry the fluffing vessels or the beaker after beginning the determination but wipe the skimmer dry each time before pouring the oysters on it. Weigh the weighing vessel and the accumulated drained oysters and calculate the weight of the latter. Wash, dry, and weigh the original container or containers and calculate the net weight of the sample. Calculate the percentage of free liquid from the difference between the net weight and the drained weight.

The laboratory studies of the development of free liquid in oysters on storage was carried on in conjunction with the work reported above on the maximum water-absorptive capacity. In these studies the oysters were drained substantially "dry" on an 8-mesh sieve, which retained variable amounts of oyster surface liquid. All the water absorption and retention data are based on the changes in weight of the oyster meats so drained. It is to be noted that such a procedure has the effect of indicating the minimum percentage of water retained and conversely the maximum percentage of free liquid developed. The free liquid developed is given in Table 10 calculated on the basis of the original weight of the oysters before any soaking treatment and on the basis of the drained weight of the oysters at the time of storage. Thus, the latter figure gives the *maximum* actual percentage of free liquid after storage. As determined by the skimmer method, these figures would be from one to two per cent lower.

It is interesting to note that the oysters can absorb much more water than they can retain. This is particularly striking in the case of the *Apalachicola* oysters, which are said to bleed excessively and which appear to retain somewhat more *absorbed* water when water is added at the time of storage.

#### *Change in Count as a Measure of Over-Run*

In Table 10 there is listed the count per quart, before washing, of successive quarts shucked without grading from the same lot of oysters. The count per quart was not made after the water treatment, but it would decrease for any single quart in proportion to the volume increase. However, it will be noted that the variation in count per quart initially was so great as to make impossible any reasonable estimate of the percentage over-run solely on the basis of the count in a quart taken at random from the lot before water treatment and the count on another quart taken after the water treatment.

TABLE 10

TYPE AND LOT NUMBER	SOLIDS	SALT	COUNT PER QUART	TREATMENT	MAXI- MUM WATER AB- SORBED	MAXI- MUM VOLUME IN- CREASE	WATER AB- SORBED AT TIME OF STORAGE	STORAGE CONDITIONS	WATER RE- TAINED	FREE LIQUID DEVELOPED	
										ON BASIS OF ORIGINAL WEIGHT	ON BASIS OF WEIGHT STORED
	per cent	per cent			per cent	per cent	per cent		per cent	per cent	per cent
Chinoteague Island, Va. 71204	19.1	1.41	—	In running water, 3.5°-5°, 21½ hours (Fig. 2)	37.2	—	—	—	—	—	—
	12.9	0.82	—	In running water, 3.5°-5°, 18½ hours	35.8	—	32.5	6 hours, room temp. (ca. 25°)	25.0	7.5	5.7
Milford Haven, Va. 71205			—	Blown 16 minutes	9.9	—	—	—	—	—	—
	14.6	0.80	—	Then in running water, 3°-5°, 21½ hours	39.0	—	28.0	46 hours, 10°	11.0	17.0	13.3
71207			67	In running water, 21°-22°, 22 hours	33.6	36	28.0	23 hours, 3°-4°	12.5	15.5	12.1
			77	In running water, 7°-9°, 22 hours	38.2	42	36.0	22 hours, 3°-4°	21.3	14.7	10.8
			74	Blown 30 minutes at 21°	21.6	28	20.8	23 hours, 10°	10.0	10.8	9.0
			78	Blown 40 minutes at 7°-9°	14.8	19	—	—	—	—	—
71208				Then in running water, 3°-4°, 16 hours (Fig. 1)	27.9	31	27.9	16 hours, 3°-4°	13.9	14.0	10.9
			68	Blown 40 minutes at 7°-9°	18.8	22	18.8	19½ hours, 10°	11.3	7.5	6.4
			73	Blown 45 minutes at 7°-9° (Fig. 3)	16.3	13	16.3	24 hours, 3°-4°	7.8	8.5	7.3
	12.8	0.83	79	Blown 40 minutes in running water, 3°	14.2	15	14.2	46 hours, 6°	7.5	6.7	5.8
Apalachicola, Florida 37313			94	In running water, 3°, 45 hours (Fig. 4)	49.2	57	49.2	Blown 20 minutes	34.2	—	—
			94	In running water, 3°, 45 hours	67.1	73	67.1	Blown 30 minutes	31.6	—	—
	12.5	0.77	94	Blown 25 minutes at 20°	12.0	15	12.0	45 hours, 0°	1.4	10.6	9.5
			103	Blown 35 minutes at 20°	9.5	10.5	9.5	Added 10.2% water; 45 hours, 0°	5.1	14.6	12.2
37314			103	In running water, 3.5°, 45 hours	55.0	56	—	—	—	—	—
			69	Blown 25 minutes at 20°	11.3	13	11.3	45 hours, 0°	2.8	8.5	7.6
	14.0	0.97	73	Blown 25 minutes at 20°	12.1	15	12.1	Added 10.0% water; 45 hours, 0°	7.1	15.0	12.3
			78	In running water, 3.5°, 45 hours	55.0	52	—	—	—	—	—

## CONCLUSIONS

(1) The variation in composition of shell oysters in a given lot is so great that the decrease in solids content on washing as determined on a sample of reasonable size cannot be relied on as a measure of the water absorbed in commercial practice.

(2) Shucking oysters into water instead of into dry pots does not decrease the loss of solids by bleeding but does make it easier to clean the oysters. It is a necessary practice for the production of adequately cleaned oysters from dirty shell stock.

(3) The quantity of water that a sample of shucked oysters will absorb under controlled conditions can not be used as an indication of the quantity they have already absorbed.

(4) The measurement of free liquid should be put on a weight basis and the entire sample should be used in the determination.

(5) Oysters that have absorbed an excessive quantity of water may develop a significant quantity of free liquid on storage.

(6) The variation in size of oysters in a lot is so great as to require very extensive studies in the field before the possibility of using the change in count per gallon as a measure of over-run could be determined. The direct measurement of over-run is just as feasible and more reliable.

## ACKNOWLEDGMENT

The writers are indebted to Miss Lila Knudsen of the Food and Drug Administration for advice and assistance in connection with the statistical aspects of the experimental work.

## A COLLABORATIVE STUDY OF THE A.O.A.C. CHICK METHOD OF ASSAY FOR VITAMIN D

By F. D. BAIRD\* and C. L. BARTHEN\*

This is a report of a collaborative study conducted by the Animal Vitamin Research Council during the fall of 1940 to obtain data on the variations that may be encountered with the A.O.A.C. method of assay for vitamin D and also on the possible causes of the variations. Certain modifications that were thought to have possibilities in lessening some of these variations were also studied. Previously, most of the collaborative investigations of this method of assay had been confined to tests on an unknown assay oil. This sort of study was also conducted in this series of experiments to obtain data from a large number of laboratories in regard

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\* Authorized by the Executive Committee of the Animal Vitamin Research Council to write a report of the Series A—1940 Collaborative Experiments and submit it for publication. The authors wish to express their appreciation to the other members of the Executive Committee who cooperated in the preparation of this report.

to the accuracy of determining the potency of an unknown when the conditions of the test are well standardized and under control.

Five experiments were conducted and 31 laboratories collaborated; 8,130 chicks were used in a total of 419 groups. At least 20 chicks were used per group, except that 10 chicks were used in each negative control group.

#### EXPERIMENTAL

Special precautions were taken to avoid any variables that might be due to the preparation of rations in individual laboratories. The ingredients used in the basal rations were carefully selected and each one was mixed thoroughly in a batch mixer. The basal rations and the rations including the oil additions (experimental group rations) were prepared by one person and then distributed to all laboratories.

The U.S.P. Reference Cod Liver Oil No. 2 was obtained from and donated by the U.S.P. Convention. The assay oil was a composite sample of equal parts of 10 commercially available fortified oils, each alleged to contain 400 A.O.A.C. chick units of vitamin D per gram. Each of 10 manufacturers sent a 4 ounce sample of oil to a central laboratory, where these samples were composited, and this composite sample was then sent to the central ration mixing plant as the assay oil.

The chicks were obtained from three sources strategically located in the east, central, and the Pacific coast areas. The laboratories were divided into three sections according to locality, and the chicks used by the laboratories in any one section were from one hatch of eggs laid by flocks of breeders fed the same ration. The chicks used by any of the laboratories in one section may be considered uniform so far as the nutrition of the breeders is concerned.

Each collaborator removed the right tibia from each chick and composited these by groups after cleaning the flesh from the bone in the usual manner. The bones were preserved in alcohol and shipped to one laboratory for extraction. All tibiae submitted by the collaborators were extracted under uniformly controlled conditions; namely, 30 hours of daytime extraction with C.P. carbon tetrachloride in a modified Soxhlet apparatus. During the night, when the apparatus was not in operation, the bones were left in the carbon tetrachloride. The tibiae were then sent to another laboratory for ashing. The bones were dried to constant weight in a vacuum oven at 100° C. with a pressure of less than 10 mm. of mercury. They were then placed in a relatively cool muffle furnace (below the ignition point) until charring was complete. The temperature was then increased to 850° C. and maintained for one hour.

The figures used in this report, referred to as bone ash, represent the percentage bone ash of the fat-free dry tibiae.

Except where specifically modified by the experimental procedure as outlined, the technic of the tentative A.O.A.C. method was followed.

The composition, chemical analyses, and vitamin content of the basal rations used were as follows:

<i>Composition of Rations</i>	
<i>A.O.A.C. Basal</i>	<i>A—1940 Basal</i>
	<i>per cent</i>
Ground Whole Yellow Corn	58.
Grey Shorts	25.
Crude Casein	12.
Tri-Calcium Phosphate	2.
Dried Brewers Yeast	2.
Iodized Salt (with $\text{MnSO}_4$ )	1.
Ground Whole Yellow Corn	40.
Ground Spring Wheat	10.
Ground Oats	10.
Wheat Bran	10.
Dried Skimmilk	10.
Crude Casein	8.
Soybean Meal	5.
Dehydrated Alfalfa Leaf Meal	5.
Steamed Bone Meal	1.3
Salt (100 pts. $\text{NaCl}$ & 1.7 pts. $\text{MnSO}_4$ )	0.7

<i>Analyses</i>	
<i>A.O.A.C. Basal</i>	<i>A—1940 Basal</i>
	<i>per cent</i>
Protein	20.26
Fat	3.52
Ash	4.80
Fiber	2.55
Moisture	10.14
Calcium	0.95
Phosphorus	0.89
	<i>p.p.m.</i>
Manganese	157.00
Carotene	4.50
Thiamin	8.00

### *Experiment I*

This experiment was conducted essentially as a check on the accuracy of the present method. The U.S.P. Reference Oil groups used in this experiment were also used by each laboratory as positive control groups for the other experiments. The following groups were used: A negative control group and three or more of the following positive control groups, which received in 100 grams of A.O.A.C. ration 5, 10, 15, 20, 25, and 30 units of vitamin D from the U.S.P. Reference Oil, respectively. The assay oil (assumed potency of 400 A.O.A.C. chick units of vitamin D) was incorporated into the basal ration at the same unit levels per 100 grams of ration as the U.S.P. Reference Oil, some laboratories using all six levels and others using only three levels, which from past experience were anticipated to produce a bone ash response in an interpretable range.

The response from graded doses of vitamin D from the U.S.P. Reference Oil and the assay oil shows, on the average, a proportional increase in bone ash as the dose of vitamin D is increased. There are some cases of

TABLE 1.—Results of Experiment I—A.O.A.C. assay  
(Percentage ash of fat-free dry tibiae, right)

UNITS D PER 100 G. RAYON	U.S.P. REFERENCE OIL										ASSAY OIL				
	0	5	10	15	20	25	30	5	10	15	20	25	30		
COLLABORATOR															
East Labs. Av.	2	34.0	35.6	40.2	41.5	42.9	42.9	45.0	35.9	40.0	41.5				
	4	36.4	39.2	42.6	44.1	44.7	45.5			43.5	43.1	44.8			
	7	30.8	36.1	40.8*	42.6*	43.3*	43.7			39.3	41.6	42.5			
	8	32.1	38.1	42.6	45.5	46.2	46.4	46.0		43.0	42.9	45.0			
	9	35.4	37.7	42.2	42.8	44.4	44.5	44.8		39.8	42.4	43.8			
	10	30.9	36.9	40.4	42.0	43.3	43.8	44.3		38.5	43.0	43.3			
	11	32.5	37.4	42.1	45.1	45.3	45.4	45.3	36.3	42.4	44.0				
	12	34.3	38.0	40.0*	43.7*	44.8*	45.0	45.7		41.6	43.5	41.9			
	15	34.3	38.4	39.8	42.6	44.5	44.2	44.1		40.0	42.8	42.4			
	16	35.0	37.8	40.9	44.2	46.3	45.0	45.7		41.9	42.6	44.7			
	Av.	33.6	37.5	41.2	43.4	44.6	44.6	45.1	36.1	41.0	42.7	43.6			
	Central Labs. Av.	21	34.9	35.1	39.6	40.9	42.0	42.4	42.6		39.1	41.2	42.2		
		22 <sup>1</sup>	34.9	36.3	39.6	41.2	43.4	42.0	36.8		43.1	41.6	43.1		
		23	34.8	36.6	40.1	41.5*	43.3*	43.3	43.7*		37.5	42.7*	43.2*		
		24	33.6	35.7	39.4	39.8	42.4	43.7	44.4			41.4	41.2	42.7	
		25 <sup>1</sup>	37.0	34.6	36.7	38.5	39.5	39.2	41.2		37.5	38.3	40.7		
28		33.0	35.8	38.2	40.4	41.6	41.0	43.0	37.3	37.3	37.8	41.1	41.4	42.7	
Av.		34.1	35.8	39.3	40.7	42.3	42.6	43.4		38.0	40.8	41.9	42.1		
Pacific Coast Labs. Av.		31	35.2		40.4	41.3	42.1				38.1	41.9	41.4		
		32	37.1	39.0*	42.6*	41.4*	43.6	43.3	43.4	38.2	40.2	42.2	43.2		
		33	38.0	41.4	43.4	44.9	44.7	46.0	45.9	40.8	41.3	44.0	44.2		
		34	35.2	37.4	40.8*	42.3*	43.7	43.4	45.1	38.2	41.6	42.0			
		35	32.9	38.2	40.2	42.6	43.0	44.4	44.1	36.6	39.5	41.5	42.4	43.5	43.3
		Av.	35.7	39.0	41.5	42.5	43.4	44.3	44.6	38.5	40.1	42.3	42.8	43.5	
		All Sections Av.	34.2	37.5	40.9	42.6	43.8	44.1	44.6	37.6	40.3	42.2	43.0	42.5	43.0

<sup>1</sup> Not figured in averages—evidently some groups of bones incorrectly identified.

\* Average % ash of two or more groups (Table 6, Experiment V).



lack of apparent significant increase of percentage bone ash as the dose of vitamin D is increased and, in a few cases, actual reversals (Tables 1 and 5). The results obtained on the assay oil are more erratic than those obtained on the U.S.P. Reference Oil.

The response curves, bone ash versus level of vitamin D furnished by the U.S.P. Reference Oil, were plotted on squared paper for each laboratory. The bone ashes obtained from the groups fed different amounts of the assay oil were superimposed on the U.S.P. Reference Oil curve and the corresponding levels of vitamin D were read directly from the projection of each point on the Reference Oil coordinate. The calculated potencies obtained are only for comparative purposes and are used as an example of the variations that may be encountered within one test conducted in a laboratory and in tests conducted between laboratories using the same U.S.P. Reference Oil, assay oil, and basal ration. The estimated potency of the assay oil varied from 200 to 760 units of vitamin D per gram based on individual levels and varied from 273 to 472 units of vitamin D per gram based on an average of the estimated potencies of the individual levels obtained from each laboratory test. The estimated potency of this assay oil, on the basis of all the tests conducted, is between 360 and 375 A.O.A.C. units of vitamin D per gram.

The maximum response in bone ash was reached, in most cases, at the 20 unit level of vitamin D per 100 grams of A.O.A.C. ration. For this reason, results obtained at the 25 or 30 unit levels are of no real significance for the estimation of potency.

It is obvious from a plotting of the average of all results obtained on the U.S.P. Reference Oil and assay oil that the type of response with these two oils is different. These averages provide evidence that the assay oil contains demonstrable quantities of vitamin D<sub>2</sub> or of some substance that produced a smaller response in chickens at the higher intake levels than the antirachitic substance predominantly present in the U.S.P. Reference Oil. When the vitamin D potency of the assay oil is estimated on the basis of such a curve, potencies of 416, 364, 368, and 336 units of vitamin D per gram, respectively, are obtained at the 5, 10, 15, and 20 unit levels.

Of considerable interest is the high bone ash obtained by some of the laboratories in the negative control groups of chicks fed the A.O.A.C. ration. It may appear that this was caused by environment, but when conditions in the laboratories that showed this high ash were checked it was apparent that environment was not the answer, because most of these laboratories were conducting additional A.O.A.C. tests concurrently in the same room with the same type of equipment, and were obtaining satisfactory bone ashes of 29-33 per cent in their negative control groups. One laboratory, which used its own method of extraction and ashing, mixed its own A.O.A.C. ration, fed it to a group of chicks from the same

TABLE 2.—Results of Experiment II—1 week depletion followed by a 2 week assay period  
(Percentage ash of fat-free dry tibiae, right)

UNITS D PER 100 G. RATION (U.S.P. REF. OIL)	REGULAR ASSAY						1 WEEK DEPLETION				
	0	5	10	15	20	25	30	0	5	10	15
COLLABORATOR											
5	33.7	38.2	41.5	43.6	44.0	45.0		34.1		38.5	41.5
11	32.5	37.4	42.1	45.1	45.3	45.4	45.3	31.4		37.0	40.6
13	37.0	38.2	43.8*	45.4*	46.9*	47.1		35.5			43.6
East Labs.											46.0
Av.	34.4	37.9	42.5	44.7	45.4	45.8		33.7		37.8	41.9
											43.1
22 <sup>1</sup>	34.9	36.3	39.6	41.2	43.4	42.0	36.8	34.5		37.3	38.5
											40.8
33	38.0	41.4	43.4	44.9	44.7	46.0	45.9	35.0		42.4	43.0
34	35.2	37.4	40.8*	42.3*	43.7	43.4	45.1	33.8	36.2	38.8	41.4
Pacific Coast											43.9
Labs. Av.	36.6	39.4	42.1	43.6	44.2	44.7	45.5	34.4		40.6	42.2
											44.6
All Sections											
Av.	35.3	38.5	42.3	44.3	44.9	45.4	45.4	34.1		38.8	41.4
											43.2

<sup>1</sup> A.O.A.C. assay results not figured in averages—evidently some groups of bones incorrectly identified.

\* Average % ash of two or more groups (Table 5, Experiment V).

hatch as that used for the collaborative studies, and obtained a bone ash of 30.6 per cent compared to 37.5 per cent (obtained in Experiment 1).

### *Experiment II*

This was a study of the effect of a one-week depletion period. Chicks were placed on the A.O.A.C. ration for one week and then selected for uniformity in weight. Some laboratories allowed a variation of only  $\pm 5$  grams from the average weight and others allowed a variation of  $\pm 10$  grams. The groups of selected chicks were given the experimental group rations containing the specified amounts of vitamin D from the U.S.P. Reference Oil for two weeks, at which time the test was terminated.

The results obtained from a one-week depletion period and selection of chicks for uniformity in weight, followed by a two-week assay period, were comparable with the results obtained on the regular three-week assay. The bone ash increased with an increased dose of vitamin D. Greater increments in bone ash than in the regular assay groups are noticeable, but consideration must be given to the fact that in the regular assay groups the maximum of response was reached with a lower dose of vitamin D than was administered to the depletion period groups. This may be accounted for by the lower total intake of vitamin D by the chicks that were subjected to a one-week depletion period before they received any vitamin D in their ration.

With the exception of the results obtained in the laboratory of Collaborator 5, the bone ashes in the negative control groups used in one-week depletion tests were lower than those used in conjunction with the regular assay, even though the total period the chicks were fed only the basal ration was three weeks in both cases. It is possible that this may be explained by the selection of the chicks after the one-week depletion period, but it may also be due entirely to variations of the chicks.

### *Experiment III*

An experiment was conducted to compare the results produced by a new basal ration (A—1940) and the A.O.A.C. ration. Groups of chicks were fed the A—1940 ration, in which was incorporated the U.S.P. Reference Oil as a source of vitamin D. The following groups were used: A negative control group and three or more of the following reference oil groups, which received per 100 grams of ration 5, 10, 15, 20, 25, and 30 units of vitamin D, respectively. For comparison, groups of chicks were fed the A.O.A.C. ration with the same amounts of U.S.P. Reference Oil incorporated.

The experimental results obtained with the A—1940 basal ration show approximately the same discrepancies that are shown with the A.O.A.C. ration. There was, however, a more uniformly graded response to increased intake of vitamin D. This may be due in part at least to the response of

TABLE 3.—Results of Experiment III—Results from new ration and A.O.A.C. ration compared  
(Percentage ash of fat-free dry tibiae, right)

(Percentage ash of rat-free dry tonnage, right)

UNITS D PER 100 G. RATION (U.S.P. REF. OIL)		A.O.A.C. BASAL RATION					A—1940 RATION								
		0	5	10	15	20	25	30	0	5	10	15	20	25	30
COLLABORATOR															
East Labs.	1	33.1	39.6	42.3*	44.1				29.6		35.7	40.5*	41.8		
	2	34.0	40.2	41.5	42.9	42.9			29.2	30.8	36.5	37.6			
	3	37.8	41.5	45.9*	45.7*	46.2			32.4		36.5	39.7*	41.5*	44.0	
	7	30.8	36.1	40.8*	42.6*	43.3*	43.7		29.5		35.9	40.3	41.5		
	10	30.9	36.9	40.4	42.0	43.3	43.8	44.3	27.9		33.7	36.8	39.0		
	11	32.5	37.4	42.1	45.1	45.3	45.4	45.3	28.9	31.3	35.8	42.9			
	12	34.3	38.0	40.0*	43.7*	44.8*	45.0	45.7	30.4		37.8	39.8	40.2		
	13	37.0	38.2	43.8*	45.4*	46.9*	47.1		32.3		38.8*	40.9*	43.3*		
	16	35.0	37.8	40.9	44.2	46.3	45.0	45.7	32.2		37.8	39.1	42.6		
Av.	33.9	37.1	41.0	43.6	44.7	44.9	45.3	30.3	2.0	36.5	39.7	41.4		11.6	
Increase in % Ash			3.2	7.1	9.7	10.8	11.0	11.4			6.2	9.4	11.1		
Central Labs.	26	35.7	41.6	42.6*	45.6	46.3			32.0		39.2	41.8*	42.3	45.0	
	27	31.8	35.8	39.6*	42.7				29.2	32.4	36.4*	39.3			
	Av.	33.8	40.6	42.7					30.6	3.2	37.8	40.6	10.3	13.0	
	Increase in % Ash		4.0	6.8	8.9	9.9	10.6				5.2	10.0			
Pacific Coast Labs. Av.	32	37.1	39.0*	42.6*	41.4*	43.6	43.3	43.4	31.1	33.9	37.0	39.2			
	33	38.0	41.4	43.4	44.9	44.7	46.0	45.9	32.4		40.5	42.0	42.9	44.6	
	34	35.2	37.4	40.8*	42.3*	43.7	43.4	45.1	31.2	35.2	36.2	40.6	40.6	41.5	
	35	32.9	38.2	40.2	42.6	43.0	44.4	44.1	29.9	31.5	33.7	38.3	39.6	40.8	
	36	33.6	39.7	42.4	42.6		43.5	43.5	29.5		36.0	39.8	40.8	43.2	
	Av.	35.4	39.0	41.3	42.7	43.5	44.3	44.4	30.8	33.5	36.7	40.0	41.0	42.8	
	Increase in % Ash		3.6	5.9	7.3	8.1	8.9	9.0		2.7	5.9	9.2	10.2	10.6	
All Sections															
Av.		34.4	37.7	41.1	43.2	44.4	44.8	44.8	30.5	32.5	36.7	39.9	41.3	42.8	
Increase in % Ash			3.3	6.7	8.8	10.0	10.4	10.4		2.0	4.2	9.4	10.8	12.3	

\* Average % ash of two or more groups (Table 5, Experiment V).

most of the groups falling in a lower bone ash range than did the groups fed the A.O.A.C. ration.

In every case a lower percentage bone ash was obtained in the negative control groups fed the A—1940 ration than in the negative control groups fed the A.O.A.C. ration. The spread between the low ash of the negative control groups and the high ash obtained in the groups fed vitamin D was greater on the A—1940 ration than on the A.O.A.C. ration. This new basal ration was compounded so as to be complete in all known essential nutritional factors for the chick except vitamin D, and the weight gains of the chicks in addition to the physical condition when noted indicate that this ration produces a more nearly normal chick than does the A.O.A.C. ration when a sufficient quantity of vitamin D is added to it.

#### *Experiment IV*

This was an experiment to study the effect of the length of the assay period on the accuracy of the test. The assay period was extended to four weeks, otherwise the A.O.A.C. procedure was followed. The results of this experiment were compared directly in the same laboratory with results obtained from the regular three-week A.O.A.C. test.

The results obtained from a four-week assay period appear to be about the same as those from the regular three-week period but the limited data do not warrant any discussion or definite conclusion.

#### *Experiment V*

This was a study conducted to obtain information on the variation to be expected between two or more groups of chicks fed the same experimental ration and getting the same intake of vitamin D.

The bone ash obtained from duplicate groups of chicks indicates very clearly the variation that may occur among like groups. It will be observed that there are approximately the same differences in percentages of bone ash between duplicate groups whether fed the A.O.A.C. ration or the new basal ration and whether the source of vitamin D was the U.S.P. Reference Oil or the assay oil. The actual difference in spread was 0.1 to 3.1 per cent bone ash and the average difference of all groups, regardless of the ration or source of vitamin D, was 1.134 per cent bone ash.

#### SUMMARY

The three sections into which the laboratories were divided did not show any apparent differences in results.

The standardization of certain procedures used in these collaborative studies did not help to eliminate variables or to improve the accuracy of the method of test over that reported in other collaborative studies, by other investigators, or that experienced by individual laboratories. As

TABLE 2.—*RESULTS OF EXPERIMENT I V—4 week assay period instead of regular 3 week assay period*  
(Percentage ash of fat-free dry tibiae, right)

UNITS D PER 100 G BAYTON (U.S.P. REF. OIL)	3 WEEK ASSAY PERIOD					4 WEEK ASSAY PERIOD								
	0	5	10	15	20	25	30	0	5	10	15	20	25	30
COLLABORATOR														
14	31.3	37.0	39.3	42.3				33.2	34.5	38.7	42.8			
24	33.6	35.7	39.4	39.8	42.4	43.7	44.4	33.6			39.6	44.4	44.6	
33	38.0	41.4	43.4	44.9	44.7	46.0	45.9	34.9		40.7	41.8	46.4		46.0

COLLABORATOR

TABLE 5.—*Experiment V—Groups of chicks fed like intakes of vitamin D*  
(Percentage ash of fat-free dry tibiae, right)

COLLABORATOR	UNITS D PER 100 G. RATION (U.S.P. REF. OIL)	% BONE ASH					DIFFERENCE		% BONE ASH		DIFFERENCE	
		A.O.A.C. Ration							A-1940 Ration			
1		43.0					1.5		39.4		2.1	
		41.5							41.5			
3		46.0			46.0		0.3	0.6	39.0	39.9		
		45.7			45.4				40.3	43.0	1.3	3.1
7		40.1			43.0		1.4	0.6				
		41.5			43.6							
12		39.6	43.1	44.9	44.9	0.7	1.1	0.3				
		40.3	44.2	44.6	44.6							
13		42.8	44.6	46.6	46.6	1.9	1.5	0.6	38.2	41.7	42.5	
		44.7	46.1	47.2	47.2				39.4	40.1	44.1	1.2 0.4 1.6
26			42.3			0.6				41.1		
			42.9							42.4		
27		39.5				0.1				37.1	1.3	
		39.6								35.7		
32	38.2	41.4	42.9			1.5	1.3	3.1				
	39.7	43.7	39.8									
34		41.3	42.9			1.5	1.3					
		40.6	41.6									
		39.9										
		41.4										
D units from		U.S.P. Ref. Oil							Assay Oil			
23		41.9	43.5						42.2	43.4		
		41.0	43.0			0.9	0.5		43.1	43.0	0.9	0.4

Average difference all groups—1.134 % bone ash.

these tests were so planned and conducted that the basal rations used by all collaborators were alike in all respects, and the extraction and ashing technics were the same, it is thought that the main cause of the variations obtained must be in the chicks themselves. Environmental conditions within a laboratory may also be an important factor. An experimental animal that will react more uniformly is needed, and this is thought possible to achieve only by developing a strain of chicks with a high vitamin D requirement and which will respond proportionately to graded doses of vitamin D. Environmental conditions will undoubtedly always vary in some respects, but those within a laboratory where a series of tests is being conducted should be uniformly controlled, especially as regards light conditions, temperature, humidity, and the absence of any ultra-violet rays.

The results of estimation of potency of the assay oil show a wide variation in each laboratory as well as among all the laboratories. The assay oil used, a composite of ten commercially available fortified oils, each of which supposedly contained 400 A.O.A.C. chick units of vitamin D per gram, showed an estimated potency of 360–375 A.O.A.C. chick units of vitamin D per gram.

Although theoretically the use of a depletion period followed by the selection of chicks should make for greater uniformity and accuracy, the data obtained from these studies do not bear out this contention, in that there was evidently no improvement in the accuracy of the test.

The use of a new basal ration, which had sufficient known nutritional essentials for the chick, with the exception of vitamin D, produced results slightly superior to those produced by the A.O.A.C. ration. Further study along this line is indicated.

The data obtained from a four-week assay period were too limited to draw any definite conclusion. However, there seems to be nothing to be gained in accuracy by lengthening the assay period over the prescribed three weeks.

The duplication of groups helped but little to achieve greater accuracy. The averaging of duplicate groups, however, did tend to smooth out the response curves. From this standpoint, the duplication of groups should help in the interpretation of the potency of an assay oil.

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## BOOK REVIEWS

**Chemistry of Food and Nutrition—6th Ed.** By H. C. SHERMAN. 611 pp. Illustrated. The Macmillan Co., New York. Price \$3.50.

To those even remotely acquainted with the field of nutrition the earlier editions of this outstanding text on the subject need no introduction. The sixth edition maintains the author's former clear-cut presentation and general organization. The chemical aspects of the subject are limited primarily to structural formulas of the various classes of foodstuffs and to rather general descriptive statements regarding the chemical reactions involved in metabolism. A good standard text in biochemistry would offer valuable supplemental information, for, as the author points out in the introductory chapter, the chief purpose of this book is to offer not the chemistry of foodstuffs but rather the nutritional function of these substances.

The text of this new edition has been revised throughout to bring the swiftly advancing subject up to date, and about half of it has been completely rewritten. This is particularly true of the chapters on vitamins, dietary standards, food economics, and nutrition as it relates to human progress. The sections on the vitamins have been taken out of their old alphabetical order and rearranged for more logical presentation of subject matter. A chapter on nicotinic acid and pellagra has been added, also one dealing with the less well-known water-soluble vitamins and "substances of related interest." The illustrations are essentially the same as those appearing in the 5th edition.

The chapter on iron and copper concludes with a general, very practical summary of the iron problem in nutrition in order to assist the student in obtaining a well-balanced conception of a subject that the author believes is too frequently presented in an alarming manner. The general organization of the chapters on ascorbic acid, thiamine, riboflavin, and vitamins A and D is essentially unchanged. The material for discussion is well chosen and well supplemented by excellent bibliographies. It is of interest to note that the author believes that thiamine can be furnished readily in adequate amounts in a natural dietary, a point which, in the opinion of the reviewer, needs particular emphasis at this time. A separate chapter devoted to the subject of reproduction and lactation, instead of including these discussions in the chapter relating to vitamin E, is a desirable change.

The last chapters are concerned with less tangible phases of the general subject. Growth in relation to calorie, protein, mineral, and vitamin intake is discussed, and the complexities and value of further researches in this field are indicated. Dietary standards, their value and limitations, and need for discretion in their use, are provocatively set forth. Another section discusses the best use of foods in order to obtain a superior organism as the result of optimal rather than merely adequate nutrition. The chapter devoted to food economics presents several problems. The question of the most valuable investments of the family budget allocated for food is recognized and discussed, and the percentage of the family income that should go for food is reconsidered. Considerable discussion is also given to the part the Government should have in assisting the individual to obtain the right foods in liberal quantities, and whether this might be attained through more extensive nutritional education or through increased purchasing power by subsidizing food distribution. In his last chapter Dr. Sherman has summarized various researches that indicate the potentialities of "buoyant" health and longevity that might be attained through a lifetime of optimum rather than maintenance level nutrition. He discusses the effects that such a nutritional program could have upon the individual, not as years added to the end of life but rather as a more extensive and more productive span, which

ultimately would reflect its economic and social benefits upon the nation. Repetitions that may seem to some as excessive in the last several chapters can be justified when one is reminded that this is a text on nutrition and that fundamental practical suggestions for optimum nutrition can bear repeating to students. The theme of these pages might be briefly stated as better nutrition through greater use of fruits, vegetables and milk or milk products.

Many will find the revised tables of the appendix particularly useful. Proximate composition and energy values of foods taken chiefly from the U. S. Department of Agriculture Circular No. 549 (1940) are included. Data on the vitamin content of foods expressed in actual weights for ascorbic acid, thiamine, and riboflavin will prove most welcome. Vitamin A values are given in International Units. In addition to revising mineral data in accord with the latest analyses, a new table of copper and manganese values for a number of common foods has been included.

The increased scope of the subject and the necessary limitations in the size of such a volume necessitate a rather abbreviated consideration of many of the topics included. Any feeling that the reader may have regarding the inadequacy of the discussion of some of these subjects is excellently compensated for by a carefully chosen and up-to-the-minute bibliography. This edition, which has been so painstakingly brought up to date, will continue to be an outstanding text in the field of nutrition.—ESTHER PETERSON DANIEL.

**Textbook of Chemistry.** By ALBERT L. ELDER, Professor of Chemistry, Syracuse University. Harper and Brothers, New York. 1941. VIII+751 pp. Price, \$3.75.

This book is designed as an introduction of general chemistry to serious students of science. It deviates from the usual form of textbook in that considerable introductory material is devoted to chemical and physical theory difficult to understand unless the reader is fairly familiar with the properties of the elements and has some basic training in physics. For example, the first 140 pages are devoted to atomic weights, isotopes, structure of the atom, radioactivity, crystal structure, and in general, concepts of physical chemistry. Following this, oxygen and hydrogen are treated in the usual descriptive way and then follows over 100 pages in which solutions, electricity, properties of ions, and chemical equilibrium are discussed. From this point the elements are treated in an orthodox manner, although at times the presentation tends toward the sensational. Finally, the subject of organic chemistry is superficially treated in about 70 pages. This, it is believed, could advantageously be condensed to a few pages and introduced under the treatment of carbon.

It is the reviewer's opinion that the organization of material as presented is pedagogically unsound. However, the book is well written, it is of good paper and print, and it is unusually well illustrated with diagrams and cuts. There is ample material present for a thorough year's course in general chemistry, but an instructor would be compelled to use judicious care in selecting topics in order to give a systematic presentation of the subject.—E. P. CLARK.

**The Spectrochemical Analysis of Metals and Alloys.** By F. TWYMAN, F.R.S. New York. 1941. Chemical Publishing Company, Inc., New York. Pp. 329 and index. Price, \$8.50.

This book includes some material published by the author in the *Practice of Spectrum Analysis* (1935) and *Spectrochemical Analysis in 1938* (1938) as well as a large amount of new data on the analysis of metals garnered from the literature and from investigations in the author's laboratory. The text is divided into two parts: the first presents a history of spectrochemical analysis, elements of atomic spectrum theory, and descriptions of spectrographic equipment; the second part covers the

excitation of spectra, general technic and a critical survey of methods for the analysis of the more common metals. The analysis of non-metallic substances occupies a short section at the end of the book. An appendix adding definitions and other notes and extensive author and subject indices follow.

The book, remarkably complete in the field of analysis of metals, includes the more recent developments in excitation, sampling, photometry, and rapid control analyses. The listing of recommended and alternative methods for the analysis of 13 metals and their alloys is a step in the direction of standardizing analytical procedures. The spectroscopist interested in the analysis of materials other than metals will find the historical introduction and discussion of theory worth reading; much of value will be derived from descriptions of instruments and technics, many of which are used in the analysis of non-metallic substances. Those contemplating installation of spectrographic equipment would do well to read Chapter VIII on Types of Problem to Which Spectrochemical Analysis Is Applicable. This section treats of applications both to metallic and non-metallic substances and is an honest survey of the present usefulness of the spectrograph.

A mild criticism is earned by the author for his failure to mention, beyond listing manufacturers' names, the extensive developments in this country in new apparatus. Grating spectrographs are dismissed (p. 69) with time-worn objections despite the availability of practical instruments of this type in America.

Many observations of the author, reflecting his practical experience, enliven the book and contribute to its sustained interest. A statement on page 158 aptly suggests that "the spectroscopist should combine a suspicious temperament with a wide knowledge of chemistry and metallurgy." This book is recommended as a valuable addition to the library of the metallurgical analyst as well as a source of general information for every practising spectroscopist.—B. F. SCRIBNER.

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